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(54) Title: MYC TARGETS

(57) Abstract: The invention relates to the treatment and diagnosis of cancer and to the development of drugs for the treatment of cancer. The invention provides a catalogue of > 350 targets of the myc oncogene family, as identified by SAGE (Serial Analysis of Gene Expression). The invention allows the analysis of myc downstream targets in view of the full context of myc induced changes in gene expression. The invention provides the insight that it is the myc oncogene family itself that provides for the recruitment and adaptation of the in essence normal physiological mechanisms and events to support the essentially neoplastic character of a cancer cell resulting in growth, invasion and spread (see fig.1). Herewith the invention provides a method for the treatment of cancer comprising modulating a myc-dependent downstream gene capable of supporting an essentially neoplastic characteristic of said cancer. The invention also provides a method to use one or more gene products of myc-downstream genes as readout in screenings assays to identify drugs interfering with myc downstream effects. The insight provided by the invention that myc boosts the cellular protein synthesis machinery can be used to optimise cellular production systems for recombinant protein synthesis systems.

Title: MYC targets

The invention relates to the treatment and diagnosis of cancer and to the development of drugs for the treatment of cancer.

The normal healthy organism maintains a carefully regulated balance that responds to specific needs of the body. In particular, the balance between the creation or multiplication of new cells and the death of superfluous cells is well maintained. However, occasionally the exquisite controls that regulate cell multiplication break down and a cell begins to grow and divide although the body has no need for further cells of its type, the cell becomes essentially neoplastic whereas there is no real need for it. When the descendants of such a cell inherit the propensity to multiply without responding to regulation, the result is a clone of cells able to expand indefinitely. Ultimately, a mass called a tumour may be formed by this clone of unwanted cells; the affected individual has developed the beginning of cancer. Neoplasias or tumours arise with great frequency, especially in older individuals, but most often pose little risk to their host because they are localised. Localised tumours are generally called benign, tumours become life-threatening if they spread through the body. Such tumours are called malignant and are a further development of cancer.

The major characteristics that differentiate malignant tumours from benign ones are their invasiveness and their spread. Malignant tumours do not remain localised and encapsulated; instead they invade surrounding tissues, get into the body's circulatory system, and set up areas of proliferation away from the site of their original appearance. The spread of tumour cells and establishment of secondary areas of growth is called metastasis; the ever multiplying and spreading malignant cells have acquired the ability to metastasise.

Because apparently benign tumours may progress to malignancy and the earliest stages of malignant tumours are hard to identify, pathologists are rarely sure how a malignancy began. In any case, the cells of malignant tumours have a tendency to lose differentiated traits, to acquire an altered

chromosomal composition, and to become essentially metastatic, they become invasive and spread.

A wealth of knowledge has been developed about the genetic events that transform a normally regulated cell into one that grows without responding to controls. These genetic events are generally not inherited through the gametes; rather they are changes in the DNA of somatic cells. The principal type of change is the alteration of pre-existing genes to oncogenes, whose products cause the inappropriate cell growth. Thus DNA alteration is at the heart of cancer induction and much focus has always been given in scientific research to elucidating the causative genetic events. For example, the members of the myc oncogene family play an important role in cancer. The frequency of genetic alterations of myc genes in human cancers (Dang and Lee, 1995) has allowed an estimation that approximately 70.000 U.S. cancer deaths per year are associated with changes in myc genes or in their expression. Three members, N-myc, c-myc and L-myc are rearranged, amplified, mutated and/or over-expressed in e.g. many cancers of lung, breast and colon, as well as in leukemia's and brain tumors.

The c-*myc* gene is expressed in a wide variety of tissues and tumors, while N-*myc* expression is largely restricted to embryonic tissues, pre-B cells and neuroendocrine tumors. N-*myc* is amplified in human neuroblastoma and small cell lung carcinoma and strongly expressed in Wilms' tumours and retinoblastoma. Neuroblastoma is a childhood tumor with a highly variable prognosis. Approximately 20% of neuroblastomas have N-*myc* amplification and these tumors follow a very aggressive course (Schwab *et al.*, 1983, Seeger *et al.*, 1985). Over-expression of transfected N-*myc* genes in neuroblastoma cell lines strongly increased proliferation rates (Bernards *et al.*, 1986, Lutz *et al.*, 1996). Transgenic mice over-expressing N-*myc* in neural crest-derived tissues showed a frequent development of neuroblastoma (Weis *et al.*,1997). Numerous comparable observations have implicated c-*myc* and L-*myc* in the pathogenesis

of many other tumor types (Cole, 1986, Marcu et al., 1992, Henrikson and Luscher, 1996).

The myc-family members are transcription factors with a basic/helix-loop-helix/leucine zipper (bHLHzip) domain. Heterodimers of myc and MAX proteins bind to the E-box motive CACGTG and activate target gene transcription (Blackwood *et al.*, 1992, Alex *et al.*, 1992; Ma *et al.*, 1993). The limited number of identified target genes thus far precluded the identification of myc downstream pathways. However, many experiments have suggested a role for *myc* genes in cell cycle control, metastasis, blocking of differentiation, apoptosis and proliferation rate (Henriksson and Luscher, 1996, Dang, 1999, Schmidt, 1999). Phenotypic analyses of mammalian cell lines and *drosophila* mutants with impaired myc function suggested a role for *myc* genes in cellular growth. Inactivation of both c-*myc* alleles in rat fibroblasts resulted in a 2- to 3-fold reduced growth rate (Mateyak *et al.*, 1997). Impaired *in vivo* expression of *drosophila* d*myc* retards cellular growth and results in adult flies half the normal size. A role for myc genes in growth regulation is furthermore in line with their effect on the cell cycle. Inactivation of c-*myc* in rat fibroblasts prolonged the G1 and G2 phases of the cell cycle, but not the S phase. High expression of c-*myc* or N-*myc* in human cells accelerated transition through the G1-phase (Steiner *et al.*, 1995; Lutz *et al.*, 1996). The same effect was found in *drosophila* cells, where reduced d*myc* activity increased the length of the G1 phase, while increased d*myc* expression enhanced transition through G1 (Johnston *et al.*, 1999).

Many studies have implicated expression of myc-family oncogenes in metastasis. In several tumor series there is a correlation between expression of myc genes and occurrence of metastases. This was observed for c-myc in e.g. breast cancer, bone tumor and colon cancer (Sierra et al., 1999, Gamberi et al., 1998, Kakisako et al., 1998). Experimental systems confirm a direct relationship between expression of myc genes and metastatic capacity. For instance human melanoma cells overexpressing c-myc were more metastatic

than control melanoma cells (Schlagbauer-Wadl et al., 1999). However, the mechanism how expression of myc genes increases the metastatic potential of tumor cells is unknown.

Several direct targets of c-myc, as well as a series of indirectly induced genes, have been identified, but no obvious links between these genes have been found. The incidental and isolated characteristics of these observations have precluded the identification of a comprehensive and integrated view of the cellular effects of myc genes in cancer. Examples are prothymosin α (Eilers *et al.*, 1991), ornithine decarboxylase (Bello-Fernandez *et al.*, 1993), the embryonically expressed ECA39 gene (Benvenisty *et al.*, 1992), translation initiation factors eIF-4E and eIF-2-alpha (Jones *et al.*,1996; Rosenwald *et al.*, 1993), the CAD gene (Boyd *et al.*, 1997), the DEAD-box gene MrDb (Grandori *et al.*, 1996) and recently nucleolin (Greasley *et al.*, 2000). Effects on cyclins and other cell cycle regulators depend on cell type and conditions. Induction of cyclin D1 or tyrosine protein phosphatase cdc25A were found in some model systems only (Galaktionov *et al.*, 1996, Amati *et al.*, 1998, Philipp *et al.*, 1994; Daksis *et al.*, 1994; Solomon *et al.*, 1995). Also induction of cyclin E and A expression has been reported (Jansen-Durr *et al.*, 1993; Hanson *et al.*, 1994). The c-*myc* targets prothymosin α and ornithine decarboxylase are also induced by N-*myc*, but it is unknown whether c-*myc* and N-*myc* share all their targets (Lutz *et al.*, 1996). Thus, it is clear that myc genetic alterations are central, and indeed, many of them have been identified and mapped.

However, much less effort has been spent on elucidating the supporting physiological events taking place in the ever multiplying and spreading cancer cell. That cells undergo genetic changes in their course to cancer is well understood. How these genetically changed cells recruit and adapt normal physiological mechanisms and events to support their essentially neoplastic character resulting in growth, invasion and spread, is much less well understood. For example, the myc proteins are transcription factors. They form dimers with the MAX protein and recognise the DNA sequence CACGTG. Very

few target genes of the myc transcription factors have been identified thus far. The identified targets do not permit a clear understanding of the pathway that is activated by myc proteins, and therefore the biochemical role of these proteins in pathogenesis is matter of much speculation. Phenotypic observations on mammalian cell lines, transgenic mice and mutant *drosophilas* with aberrant expression of myc genes has suggested a role for myc genes in cell cycle control, metastasis, apoptosis, proliferation rate and cellular growth. The present invention discloses at least 7 groups of myc regulated genes.

The invention now provides the insight that it is the myc oncogene family itself that provides for the recruitment and adaptation of the in essence normal physiological mechanisms and events to support the essentially neoplastic character of a cancerous cell resulting in growth, invasion and spread. The invention provides a nucleic acid library comprising *myc*-dependent downstream genes or functional fragments thereof said genes essentially capable of supporting a neoplastic character of cancer such as growth, invasion or spread. A nucleic acid library is herein defined as a collection of nucleic acid sequences comprising genes or functional fragments thereof which are downstream myc-dependent genes or functional fragments thereof. Up- or down-regulation of these genes is at least dependent on the presence of transcription factors encoded by the myc-family members. In such a collection of nucleic acid sequences the sequences are preferably available in a vector which provides easy handling of the collection of nucleic acid sequences. The downstream genes or functional fragments thereof of the myc genes were identified by applying the SAGE (Serial Analysis of Gene Expression) technique. Initially, about 66,233 tags were identified, each representing a mRNA transcript, in a pair of N-myc transfected and control-transfected neuroblastoma cell lines. Thus, we have identified 197 tags, each representing a transcript, that are specifically induced and 85 tags that are

suppressed by N-myc (table 1). In an extension of these analysis to 79,100 transcripts, we identified an additional series of transcripts that are up- or down-regulated by N-myc (table 2).

Functional fragment is herein defined as a part of a myc-dependent downstream gene which still contains the appropriate Tag-sequence to provide identification by the SAGE technique.

In particular, the invention provides a nucleic acid library wherein said *myc*-dependent downstream genes each comprises a nucleic acid essentially equivalent to a Tag sequence as shown in Table 1 or Table 2. Essentially equivalent herein relates to Tag sequences that identify similar or related genes or fragments thereof. In a more preferred embodiment a nucleic acid library according to the invention is provided wherein said myc-dependent downstream genes encode a ribosomal protein, a protein related to protein synthesis, a protein related to metastasis, a glycolysis enzyme or a mitochondrial functional protein.

Herewith the invention provides a method for the treatment of cancer comprising modulating a *myc*-dependent downstream gene capable of supporting an essentially neoplastic character of said cancer, such as growth, invasion or spread. In particular, the invention provides a method wherein said *myc*-dependent downstream gene comprises a nucleic acid essentially equivalent to one of a Tag sequence as shown in Table 1 or Table 2. Essentially equivalent herein relates to Tag sequences that identify similar or related genes or fragments thereof.

For example, the invention provides downstream genes that are activated or repressed by N-*myc* in human neuroblastoma. The analysis of the expression level of more than 66,233 transcripts identified 199 up-regulated and 85 down-regulated transcripts in N-myc expressing cells (Table 1) (Boon et al. 2001). An extension of these analysis to 79,100 transcripts identified an additional series of transcript tags that are up- or down-regulated by N-myc (Table 2). Our results show that N-myc functions as a regulator of cell growth,

facilitating neoplasia, by stimulating genes functioning in ribosome biogenesis and protein synthesis, as well as in mitochondrial electron transfer and ATP synthesis. Furthermore, many genes involved in cell architecture and cell-matrix interactions are down-regulated, facilitating invasion or spread of the neoplastic cell. A series of the N-myc regulated genes are target of c-myc regulation as well.

The invention herewith provides a list of MYC-family target genes with a large number of identified targets which permits the identification of pathways induced or inhibited by myc-family genes. However, with the identification of individual genes several other practical applications are provided as well. The findings can be used for e.g. development of new drugs, refined use of known drugs and recombinant technology. Some examples are described below.

For example, the invention provides assays for high-throughput screening of drugs specifically inhibiting myc-proteins or myc-downstream pathway proteins. It is well established that an individual cancer is caused by mutations in several oncogenes and/or tumour suppressor genes. Tumors of one and the same tissue can arise from different combinations of mutated oncogenes and tumor suppressor genes. The type and combination of gene defects determine the biology of the tumor cells, and thus the clinical behavior of the tumor. Future tumor therapies will be tailor made for the tumor of individual patients. Upon diagnosis, the type of oncogene activations in a tumor will be established and this will guide the choice of therapy. About 70.000 cancer deaths per year in the U.S. are associated with defects in myc genes or over-expression of these genes (Dang and Lee 1995, Dang, 1999). There are presently no drugs specifically blocking the action of myc proteins. Such drugs can now be identified in high-throughput systems, using target genes as provided by the invention, where thousands of compounds can be tested for a specific inhibitory effect on myc proteins. These test-systems need a very strict read-out system. An example of such a system used to identify inhibitors of the TP53 tumor suppressor protein was recently published

(Komarov et al., 1999). A lacZ reporter construct was brought under the control of a promoter of a gene known to be induced by TP53. A mouse cell line harboring this construct was used to test 10.000 synthetic chemical compounds. The compounds were added to tissue culture wells with these cells and the few compounds inactivating TP53 were identified by a reduced expression of the LacZ reporter gene.

Our identification of the series of myc-target genes enables a sophisticated approach to identify myc-inhibitory drugs. There is presently no sensitive read-out system to identify such drugs. Some target genes of c-myc and/or N-myc have been identified, but their expression levels are only a few times modulated, which makes them essentially useless as read-out system (reviewed in Cole et al., 1999). Here we describe the identification of a series of genes that are strongly induced or suppressed by N-myc and/or c-myc. For example we showed that expression of the osteonectin or SPARC gene is down-modulated by N-myc from 280 transcripts per 10.000 transcripts in SHEP-2 to 14 transcripts in SHEP-21N. This 20-fold reduction is fully confirmed by Northern blot hybridization of mRNA from SHEP-2 and SHEP-21N (data not shown). Moreover, we confirmed that also at the protein level, SHEP-2 cells have much more Osteonectin protein than SHEP-2 (data not shown). The Osteonectin protein is known as a secreted protein (reviewed Lane and Sage, 1994). This makes testing of N-myc inhibitory drugs very feasible. Test-compounds can be added to SHEP cells in which N-myc expression can be induced, e.g. by a tetracyclin-inducible system. When N-myc expression is induced, Osteonectin protein levels will drop in these cells. If a compound is inhibitory for N-myc, this will block down-modulation of Osteonectin mRNA expression. These cells will continue secretion of Osteonectin protein in the tissue culture medium. Therefore, in a multi-well cell culture system, all wells with inactive compounds will have low Osteonectin production. The rare wells with a high Osteonectin concentration identify the compounds that inhibit N-myc functioning. Elisa or other protein-based detection systems can

automatically detect the wells with high Osteonectin concentrations. Since not only Osteonectin, but many other down-regulated genes like IGFBP7, collagen type 4a1 and syndecan 2 are identified to be down-regulated by myc, they can be used in such assays as well. Similar assays can be designed the other way around, using genes induced by N-myc or c-myc as a read-out. Variants of such drug-testing systems can use the promotor elements of strongly N-myc-regulated genes and use them to control expression of easily detectable proteins like Green Fluorescent Protein. This will further simplify detection of myc-inhibitory drugs in high throughput systems.

Since we showed that many of the N-myc targets are targets of c-myc as well, our findings are used in assays for the identification of drugs against other members of the myc oncogene family. Inducible c-myc constructs will in many cell systems target the same genes as N-myc. Therefore the here described list of target genes for N-myc and c-myc enables the semi-automatic testing of hundreds of thousands candidate drugs and the selection of compounds that are active against myc proteins and/or their downstream pathways.

The invention also provides the application and further development of existing drugs for specific treatment of patients with N-myc or c-myc activated tumours, for example in a method of treatment for myc-related cancer. As explained, future cancer therapies will be designed to specifically inhibit those oncogenes that are actually activated in the tumor of a specific patient. Our finding provides the deliberate use of several currently known cytostatic or cytotoxic drugs in patients with a tumor caused by activation of a myc gene. A few examples are given. For example, the invention provides a method of treatment comprising using drugs interfering with nucleophosmin:

We identified nucleophosmin (B23) as a major target of N-myc and c-myc induction. Analysis of a large series of human tumours and cell lines shows that nucleophosmin mRNA levels also *in vivo* correlate with N-myc or c-myc expression. Nucleophosmin functions in ribosome biogenesis and nucleolar-cytoplasmic transport of pre-ribosomal particles. The protein is known to be

translocated from the nucleolus to the nucleoplasm by several cytotoxic drugs, like actinomycin D, doxorubicin, mitomycin, toyocamycin, tubercidin, sangivamycin and mycophenolic acid (Yung et al., 1995, 1990, Chan et al., 1987, Chan 1988, 1992, Cohen and Glazer, 1985, Perlaky et al., 1997). Treatment of cells with these drugs inhibits processing of ribosomal RNA and protein synthesis and leads to cell death. Several of these drugs are clinically tested and/or applied for treatment of cancer patients.

Our finding that members of the myc oncogene family induce nucleophosmin mRNA and protein expression, marks this protein as an attractive target in the treatment of tumours with overexpression of a myc family member. This opens fundamental new opportunities for the above mentioned drugs and their analogons. These drugs were clinically tested or used on unselected patient series, without knowledge of their possible specific effect on myc-activated tumors. The patient series presumably consisted of patients with and without involvement of myc genes in their tumours. Actinomycin D is used in e.g. Wilms' tumor and rhabdomyosarcoma treatment. Some patients react better on therapy than other patients. A sizable part of Wilms' tumor and rhabdomyosarcoma patients have high N-myc or c-myc expression (Nisen et al., 1986). It can now be tested whether actinomycin D is specifically effective against N-myc or c-myc expressing tumors, wherein actinomycin D is ineffective against tumours that have no myc activations. These findings provide a more specific use of actinomycin D. Several other drugs are effective against nucleophosmin (see above). Improvement of these drugs by development of less toxic analogs that have more specific anti-nucleophosmin effects is now an interesting strategy provided here to design specific anti-myc drugs. E.g. toyocamycin is highly toxic (Wilson, 1968), but analogs can be tested and further developed for specific use in patients with myc-activated tumors.

The invention also provides use of inhibitors of extracellular and transmembrane proteins. The list of genes induced by N-myc and/or c-myc

includes many genes coding for secreted or cell surface proteins. Such proteins are excellent targets for drugs, as they are readily accessible. They may offer targets to specifically inhibit growth or metastasis of tumour cells with an activation of myc-family members. Examples of potential targets are basigin and Plasminogen Activator Inhibitor type 1(PAI)(Table 1, No. 112). Basigin (or EMMPRIN, extracellular matrix metalloproteinase inducer)(table 1, No. 97), is a member of the immunoglobulin family that is present on the surface of tumor cells and stimulates nearby fibroblasts to synthesize matrix metalloproteinases (Guo et al., 1998). Since metalloproteinases are known to promote degradation of matrix and promote metastasis, drugs that inhibit basigin would be able to prevent metastasis of tumors with high expression of myc-family genes. Drugs inhibiting metalloproteinase are known and could now be applied for specific treatment of tumors with activation of myc-family members.

Plasminogen activator inhibitor type-1 (PAI-1) is a major physiological inhibitor of fibrinolysis and matrix turnover. The here-described down-modulation of PAI-1 by N-myc would increase matrix turnover and promote cell motility ands metastasis. Many compounds are clinically and experimentally tested for regulation of PAI. For example 15-deoxy-Delta12, 14-prostaglandin J2 (15d-PGJ2), an activating ligand for the transcription factor PPARg, augmented PAI-1 mRNA and protein expression (Marx et al., 1999). It is here provided that these drugs are therefore specifically be used to prevent metastasis of N-myc or c-myc expressing tumors. Furthermore, transmembrane proteins induced by N-myc and/or c-myc proteins can be used as target of therapeutic drugs like antibodies that can be conjugated with cytotoxic drugs.

Furthermore, the invention provides further molecular diagnosis of tumors. Here is provided a first integral description of target genes of myc-family oncogenes. Several functional categories provided. However, in the analysis of fresh tumors with N-myc activation, we observed that not all these

genes or all categories are up-regulated in all tumors. This suggests that additional defects or factors may affect the range of genes that are induced or repressed by myc oncogenes in individual tumors. This is likely to be of importance for the biology of the tumor. Therefore, the detailed analysis of up-regulated/suppressed genes in tumours with activation of a myc-family member is clinically relevant. A tumour with up-regulation of genes involved in protein synthesis may differ from a tumor with up-regulation of genes involved in oxidative phosphorylation. This information is important to select the appropriate treatment modality for a tumor. The full list of N-myc and/or c-myc downstream targets therefore provides an important means to classify tumors for optimal therapeutic regimens. The here-described finding can be applied to develop diagnostic kits to measure the activation or inactivation of key-downstream targets of myc-family oncogenes. Such diagnostic tools are now provided to guide the optimal therapy.

Furthermore, the invention provides non-invasive diagnosis of tumor. Activation of specific oncogenes can presently only be established by analysis of a surgically removed tumor specimen. As surgery is a burden to the patient, expensive and not without risk, non-invasive methods to monitor oncogene status of tumors would be desirable. The inventory of genes for secreted proteins that are induced or suppressed by N-myc can be used to determine the status of myc expression by analysis of serum markers. Furthermore, serum levels of these genes can be used to monitor tumor growth, reaction on therapy and occurrence of relapses. Our results shows that many candidate proteins, e.g. osteonectin (reduced from 280 to 14 tags/10.000 tags), macrophage migration inhibitory factor table 1, No. 92)(induced from 1.1 to 14.4 tags/10,000 tags) and Plasminogen activator inhibitor type 1 (PAI-1) as serum markers to aid in the biological classification of tumors. Recently serum levels of PAI-1 were analyzed in a series head and neck tumors and found to correlate with tumor stage (Strojan et al., 1998). Our findings therefore identify PAI-1 and other secreted proteins affected by myc-family members as

good candidates to monitor the status of myc genes in a tumor and to follow the growth and response on therapy of myc-induced tumors.

The invention furthermore provides enhancement of cellular protein synthesis machinery for production purposes. Eukaryotic cells can be used to produce recombinant proteins, e.g. of drugs. The discovery that N-myc and c-myc induce essential components of the protein synthesis machinery can be applied to boost production of recombinant proteins in cell systems. The invention provides the use of cells with a high expression of endogenous or transfected myc genes to optimize the yields of recombinant proteins, like antibodies, hormones or other proteins with a therapeutic or commercial value.

The invention further provides a method to identify a substance capable of interfering with n-myc or n-myc induced modulation of transcripts and/or proteins, comprising providing a cell with n-myc activity or a nucleic acid encoding n-myc activity and determining the modulation of said transcripts and/or proteins in the presence of said substances. As disclosed herein the modulation of transcripts and/or proteins by n-myc can be either up- or down-regulated.

The invention will be explained in more detail in the following description, which is not limiting the invention.

Detailed description

The members of the myc oncogene family play an important role in cancer. The frequency of genetic alterations of myc genes in human cancers (Dang and Lee, 1995) has allowed an estimation that approximately 70.000 U.S. cancer deaths per year are associated with changes in myc genes or in their expression. Three members, N-myc, c-myc and L-myc are rearranged, amplified, mutated and/or over-expressed in e.g. many cancers of lung, breast and colon, as well as in leukemia's and brain tumors. The myc proteins are

transcription factors. They form dimers with the MAX protein and recognize the DNA sequence CACGTG. Very few target genes of the myc transcription factors have been identified thus far. The identified targets do not permit a clear understanding of the pathway that is activated by myc proteins, and therefore the biochemical role of these proteins in pathogenesis is matter of much speculation. Phenotypic observations on mammalian cell lines, transgenic mice and mutant *drosophilas* with aberrant expression of myc genes has suggested a role for myc genes in cell cycle control, metastasis, apoptosis, proliferation rate and cellular growth.

In order to identify the downstream pathways of the myc genes, we applied the SAGE (Serial Analysis of Gene Expression) technique. Initially, we identified about 66,233 tags, each representing a mRNA transcript, in a pair of N-myc transfected and control-transfected neuroblastoma cell lines. Thus, we have identified 197 tags, each representing a transcript, that are specifically induced and 85 tags that are suppressed by N-myc (table 1). In an extension of these analysis to 79,100 transcripts, we identified an additional series of transcripts that are up- or down-regulated by N-myc (table 2). N-myc appears to induce the expression of many ribosomal protein genes, genes involved in ribosomal RNA synthesis and ribosome biogenesis and genes involved in translation and protein maturation. This indicates that a major function of N-myc is the enhancement of the protein synthesis machinery of the cell. Furthermore, there is a striking induction of genes involved in glycolysis and in electron transport and ATP synthesis in the mitochondria. This suggests an increased capacity of the cellular energy production mechanism. Another set of N-myc targets is involved in cellular adhesion, matrix formation, invasive capacity and cytoskeletal architecture. These data explain the increased metastatic potential associated with myc-expressing tumor cells. Furthermore, a set of genes is induced or suppressed with a role in transcription, chromosome condensation and signal transduction. Finally, a series of genes are identified for which only a short cDNA sequence is known (Ests) and some

SAGE transript tags were identified without a gene assignement. These genes may be important components of the N-myc downstream pathway. Many of these downstream targets of N-myc appear to be targets of the c-myc oncogene as well. Therefore, these data represent an inventory of the target genes of the myc oncogene family. As these genes mediate the tumorigenic effects of myc-family oncogenes, they offer the opportunity to identify new drugs that inhibit myc proteins or myc downstream pathways. Furthermore, they represent a range of potential target genes to inhibit or kill tumor cells expressing members of the myc-family of oncogenes.

Results

SAGE libraries of N-*myc* transfected neuroblastoma cell lines

To identify the downstream target genes of N-myc, we applied the SAGE technique on an N-*myc* transfected neuroblastoma cell line. The SHEP cell line has no N-*myc* amplification and expression, nor c-*myc* expression. A tetracycline-dependent N-*myc* expression vector has been introduced in these cells, resulting in the SHEP-21N clone (Lutz *et al.*, 1996). The SHEP-21N cells have constitutive exogenous N-*myc* expression that can be switched off by tetracycline. N-*myc* expression in the SHEP-21N cells was shown to increase the rate of cell division, shorten the G1 phase of the cell cycle and to render the cells more susceptible to apoptotic triggers (Lutz *et al.*, 1996; Fulda *et al.*, 1999). Two SAGE libraries were constructed, one from SHEP-21N cells expressing N-*myc* and one from the SHEP-2 control cells. The SHEP-2 clone was transfected with the empty expression vector. From SHEP-2 we sequenced about 44,674 transcript tags and from the SHEP-21N library we sequenced 21.559 transcript tags. Comparison of the two SAGE libraries yielded 199 significantly ($p<0.01$) up-regulated tags in N-*myc* expressing cells, with induction levels of up to 47-fold (Table 1, section 1). Another 85 tags were significantly down regulated. Further sequencing of the SHEP-21N library

from 21,559 tags to 34,426 tags yielded another series of transcript tags that were either up- or down-regulated by N-myc ($p < 0.01$) (Table 2). Here we describe these tags and the most likely gene assignment that can presently be made. The transcripts corresponding to these tags were identified using a computer program developed by us (Caron et al., 2001) and using the SAGEmap database from CGAP/NCBI (Lal *et al.*, 1999). Seven groups of N-myc regulated genes are described.

N-myc targets 1: ribosomal protein genes

The first functional group consists of 61 ribosomal protein genes, that were induced up to 47-fold ($p<0.01$, Table 1, section1). These 61 proteins represent about 75% of the human ribosomal proteins (Wool *et al.*, 1996). Seven of the induced genes were selected for further analysis. Northern blots with equal amounts of total RNA from SHEP-2 and SHEP-21N cells were hybridized with probes for the ribosomal proteins S12, S27, Fau-S30, L8, S6, S19 and the ribosomal phosphoprotein P0 (PPARP0) (Figure 1). All seven genes were induced by N-*myc*. The total amount of tags found for ribosomal protein mRNAs comprises about 4% of all tags in SHEP-2. This fraction has increased to 10% in SHEP-21N. The level of induction of individual ribosomal protein genes is a function of their basal expression levels in SHEP-2. Highly expressed genes are less induced than genes with a low basic expression in SHEP-2 (Figure 2A). These results indicate that N-myc induces, directly or indirectly, the mRNA expression level of the majority of ribosomal proteins.

N-myc targets 2: genes functioning in ribosome biosynthesis and protein synthesis

Also a second functional group of 26 tags corresponds to genes with a distinct role in protein synthesis and turnover, notably ribosome biogenesis, mRNA translation, protein maturation and degradation.

Highly interesting is the induction of nucleophosmin (B23) (table 1, no.'s 67 and 83). Northern blot analysis confirmed this induction (Figure 1), to a level even stronger than suggested by the tag frequencies. Nucleophosmin is a highly abundant nucleolar protein that processes ribosomal RNA by cleavage of the 5' end of the 5.8S pre-rRNA (Savkur *et al.*, 1998). It furthermore functions in assembly and nuclear-cytoplasmic shuttling of pre-ribosomal particles (Borer *et al.*, 1989; Olson *et al.*, 1991, Szebeni *et al.*, 1999). Nucleophosmin is the target of recurrent chromosomal translocations in lymphomas and leukemia (Morris *et al.*, 1994, Redner *et al.*, 1996, Pandolfi, 1996). The prominent role of nucleophosmin in ribosome biogenesis urged us to analyze the SAGE libraries for other genes implicated in this process. Nucleolin, which also has two tags due to alternative transcripts (table 1, no.'s 87 and 88), is induced from 2.5 to 5.6 tags per 10,000 in total (p=0.044). This induction was confirmed by Northern blot analysis (Figure 1). Nucleolin is also a highly abundant nucleolar protein and binds to nucleophosmin (reviewed Tujeta and Tujeta, 1998; Ginisty *et al.*, 1999). It probably is a rate-limiting enzyme for the first step in the processing of the pre-ribosomal RNA to mature 18S rRNA (Gistiny *et al.*, 1998). Nucleolin is furthermore involved in the assembly of pre-ribosomal particles and their nucleo-cytoplasmic transport. It interacts with 18 ribosomal proteins (Bouvet *et al.*, 1998), sixteen of which are induced by N-*myc*. The induction of nucleolin and nucleophosmin by N-*myc* suggests that besides ribosomal proteins, also ribosomal RNA and ribosome biosynthesis are target of N-*myc* stimulation.

Also tags corresponding to three translation initiation factors and five translation elongation factors were induced. The initiation factors are eukaryotic translation initiation factor 3 subunit 8 (eIF3s8) (Table 1, No.81) and subunit 3 (Table 1, No.78), eukaryotic translation initiation factor 4B (Table 1, No.72). Elongation Factor 1 (EEF1), responsible for delivery of aminoacyl-tRNA to the ribosome, is a heterotrimer either consisting of the subunits alpha/beta/gamma or alpha/delta/gamma. The tags for the subunits

alpha, delta and gamma are induced 9- to 11.4-fold in SHEP-21N (Table 1, No.'s 69, 66 and 70). Elongation Factor 2, which promotes the translocation of the nascent polypeptide chain from the A- to the P-site of the ribosome, is also induced (Table 1, No. 79). The mitochondrial elongation factor Tu (tuFM), which delivers aminoacyl-tRNA to the mitochondrial ribosomes, is 12.4 times up-regulated (Table 1, No. 64). Northern blot analysis of SHEP-21N and SHEP-2 confirmed the induction of eIF3s8, EEF1a1 and tuFM (Figure 1). These data further support a role for N-*myc* as a regulator of protein synthesis.

A next step in protein synthesis is maturation and routing. The nascent polypeptide-associated complex (NAC) alpha mRNA was induced in N-*myc* expressing cells (Table 1, No. 77). NAC protects nascent polypeptide chains of cytosolic proteins from inappropriate translocation to the endoplasmatic reticulum (Wiedmann *et al.*, 1994). Induction of the chaperones HSP60 and HSP90 further suggested an increased cellular capacity for protein folding and maturation (Table 1, No.'s 65, 68, 80 and 82). HSP60 is implicated in mitochondrial protein import and macromolecular assembly. HSP90 is involved in the folding of a signaling molecules including steroid-hormone receptors and kinases and the refolding of misfolded proteins. Northern blot analysis confirmed the induction of HSP60 (Figure 1). Also the cellular capacity for protein degradation was possibly induced. This was suggested by the increased tag frequencies for three ubiquitin pathway proteins (Table 1, No.'s 62, 73 and 76) and five proteasome subunits (Table 1, No.'s 63, 71, 74, 75 and 84). Northern blot analysis confirmed the higher expression level of proteasome subunit b type 6 in SHEP-21N cells (Figure 1).

N-myc targets 3: glycolysis genes

A third group of N-*myc* induced genes encoded key-enzymes in the glycolytic pathway (Table 1, section 4). Tags for aldolase A fructose-biphosphate (ALDOA), triosephosphate isomerase 1 (TPI1), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and pyruvate kinase are all increased (Table 1, No.'s

133, 135 and 132). Other induced mRNAs encode for the metabolic enzymes 3-phosphoglycerate dehydrogenase, involved in the synthesis of serine, and sorbitol dehydrogenase that oxidizes sorbitol to fructose. Aldehyde dehydrogenase 1 functions in ethanol metabolism. Northern blot analysis confirmed the mRNA induction of ALDOA, pyruvate kinase, TPI1 and GAPDH (Figure 1). These data implicate the glycolysis as a target of N-myc stimulation.

N-myc targets 4: Mitochondrial electron carriers and ATP synthethase

SHEP-21N shows induction of a series of tags corresponding to genes with a role in oxidative phosphorylation in the mitochondria (Table 1 section 5). Seventeen tags are significantly induced. Interestingly, five of the induced genes are mitochondrially-encoded (see for mitochondrial tag analysis Welle *et al.*, 1999).

The oxidation of NADH and FADH2 by electron transfer to O2 is performed by three protein complexes of the respiratory chain, NADH-dehydrogenase, ubiquinol-cytochrome c reductase and cytochrome c oxidase. These large complexes establish a proton gradient across the mitochondrial inner membrane, which drives the synthesis of ATP by the F-type ATP synthase complex. N-*myc* induces a series of subunits of all four enzyme complexes.

Four NADH dehydrogenase subunits, subcomplex 4 (Table 1, No. 136; NDUFB4), subcomplex 7 (Table 1, No. 138) and the mitochondrially encoded subunits 4/4l and 3 (Table 1, No.'s 150 and 151) are induced. The induction of NDUFB4 was confirmed by Northern blot analysis (Figure 1).

One subunit of the ubiquinol-cytochrome c reductase complex was induced (Table 1, No. 136). Furthermore, subunits II, III and VIII of cytochrome c oxidase were induced in N-*myc* expressing cells. Induction of the subunit VIII (COXVIII, Table 1, No. 149) was confirmed by Northern blot analysis.

Finally, N-myc induces the transcripts of subunits 6/8 of the F0 segment and of two isoforms of subunit 9 (or c) of the F0 segment of the stalk of the F-type ATP synthase (Table 1, No.'s 137, 148, and 152). ATPase subunits 6 and 8 are encoded on an overlapping mitochondrial transcript.

In addition, several other proteins with a role in mitochondrial function are up-regulated (table 1 section 5). The voltage dependent anion channel (VDAC, Table 1, No. 143) was induced 5-fold, which was confirmed by Northern blot analysis (Figure 1). VDAC forms a mitochondrial outer membrane channel that allows diffusion of small hydrophylic molecules. It plays a major role in apoptosis, as it can transfer cytochrome c to the cytoplasm, which results in caspase 9 activation.

In addition, glutathion peroxidase 4 and glutathion S-transferase p are strongly induced (Table 1, No.'s 139 and 144). Glutathion readily accepts electrons and may serve as scavenger for hydrogen peroxide and organic peroxides, the inevitable artifacts produced by the electron transport chain of the mitochondria. This reaction is catalyzed by gluthatione peroxidase.

N-myc targets 5: genes with a role in cell motility and metastasis:

A large group of tags that are either induced or suppressed by N-myc belong to genes with a role in cell motility and cell-matrix interactions (table 1, section 3). These genes encode for cytoskeletal proteins, cell surface proteins, adhesion molecules and extracellularly secreted proteins with a role in cellular matrix architecture and turn-over. Ten tags for genes in this category were significantly induced. Another 29 tags in this category are significantly down-modulated. Examples of down-regulated genes are Collagen types Ia1 (Table 1, No.'s 100, 108 and 109), type IVa1 (Table 1, No. 115) and type XVIIIa1 (Table 1, No. 116), fibrillin (Table 1, No. 121), syndecan 2 (Table 1, No. 126), fibronectin (Table 1, No. 122) and Osteonectin (SPARC)(Table 1, No.'s 118, 123 and 125). The latter is an interesting gene, that is down-modulated from 280

to 14 tags per 10.000 tags. We confirmed down-modulation of Ostenectin, syndecan 2 and collagen IVa1 and Plasminogen activator inhibitor type 1 (Table 1, No. 112) by Northern blot analysis (data not shown). The down-modulation of these genes suggests that N-myc can reduce the adherence of cells to the cellular matrix and therefore induce the motility of the cells. This is line with an enhanced metastatic potential of myc expressing tumor cells.

N-myc targets 6: other genes.

Another group of genes that is affected by N-myc is formed by signal transduction proteins, transcription factors, chromatin factors, cyclins and other regulatory proteins. This group counts 66 significantly induced transcripts (Table 1 section 6). Examples are NM23A, NM23B, HMG I-Y, zinc finger protein 6 and (Table 1, No.'s 171, 214, 162, 218). Induction of HMG I-Y, NM23A and NM23B was confirmed by Northern blot analysis (data not shown). Another group of genes for regulatory proteins or enzymes were downmodulated by N-myc (table 1, section 6). Examples are Insulin-like growth factor binding protein 7 (IGFBP7)(Table 1, No. 252) and zinc-finger protein 216 (Table 1, No. 248). Northern blot analysis confirmed downregulation of IGFBP7 in SHEP-2 cells compared to SHEP-21N cells (data not shown).

N-myc targets 7: Anonymous genes (Ests)

A series of anonymous genes for which only a partial cDNA sequence is known (expressed sequence tags or Ests) are induced or down-modulated by N-myc (Table 1 section 7). The function of these genes is as yet unknown, but the finding that they are targets of myc regulation mark them as potentially important genes with a role in cancer.

Tags of unidentified targets of N-myc.

For several tags that were differentially represented in the SHEP-2 and SHEP-21N libraries, we have as yet not identified the corresponding genes (table 1, section 8). They belong both to genes that are induced or suppressed by N-myc.

N-myc activates downstream targets within 4 hours

In a time-course experiment we analyzed whether the putative N-myc targets are induced after N-myc modulation in the SHEP-21N system. N-myc expression can be reversibly switched-off in SHEP-21N cells by tetracycline. SHEP-21N cells were treated for 24 hours with tetracycline, washed extensively and grown for an additional 2 to 36 hours without tetracycline. Northern blot analysis showed that the expression of N-*myc* mRNA is switched off within 8 hours of tetracycline treatment (Figure 3A, lanes 1-2). After removal of tetracyclin, N-*myc* mRNA expression is restored between 2 and 4 hours (Figure 3A, lanes 5-6). The N-myc protein expression was analyzed by Western blotting in a parallel time-course experiment and closely followed the N-*myc* mRNA expression (Figure 3B). The Northern blot filters were hybridized with probes for the N-myc downstream targets nucleolin, nucleophosmin and the ribosomal protein genes RPS6 and RPS12 (Figure 3A). After repression of N-*myc* by tetracyclin, the mRNA levels of these genes remain unaffected at 0 and 8 hrs, but their expression is reduced to low basic levels at 24 hours. Importantly, between 2 and 4 hours after re-expression of N-*myc* mRNA and protein, expression of all four genes is strongly re-induced (Figure 3B, lanes 6-7). Similar results were obtained for EEF1A1, TPI1, eIF3s8, and VDAC (data not shown). The expression level of cofilin that we used as a control does not change significantly during the time course. To exclude a direct effect of tetracyclin on nucleolin or nucleophosmin expression, we did the same experiment with SHEP-2 cells but no effect on gene expression was observed (data not shown). These results firstly confirm that

the here identified genes indeed are induced by N-myc. Secondly, it shows that they are early targets in the N-myc downstream pathway, although not necessarily direct targets of N-myc. They therefore represent essential components of the N-myc pathway. The data furthermore show that the induction by N-myc is highly versatile: expression drops after N-myc abrogation and is swiftly restored after N-myc re-expression.

N-myc induces ribosomal RNA synthesis

The induction of two genes with a key role in rRNA processing and ribosome biogenesis urged us to analyze their protein expression level and their possible functional activity. Protein expression of nucleolin and nucleophosmin was analyzed in SHEP-2 and SHEP-21N cells, as well as in two control cell lines with and without N-*myc* amplification. Western blot analyses showed a higher nucleolin and nucleophosmin expression in SHEP-21N compared to SHEP-2 (figure 4, lanes 3 and 4) and in the N-*myc* amplified IMR32 cell line compared to the N-*myc* single copy cell line SK-N-FI (Figure 4, lanes 1 and 2). As these proteins function in ribosomal RNA processing, we analyzed whether SHEP-21N has a higher rRNA content than SHEP-2 cells. We isolated total RNA from 10 samples of 10^6 exponentially growing cells of each of the cell lines. Spectrophotometric analysis revealed that SHEP-21N cells have an at average 45% higher yield of total RNA than SHEP-2 cells ($p<0.001$, Student T test for independent samples) (Figure 4B). Duplicate experiments on independently cultured cells gave the same results. Densitometric quantification of the 18S and 24S rRNA bands fractionated by agarose gel electrophoresis confirmed that this increase is caused by ribosomal RNA (data not shown).

To analyze whether this strong increase in rRNA resulted in increased ribosomal function and overall protein synthesis, we measured protein content and the rate of protein synthesis in SHEP-2 and SHEP-21N cells. Lysates of 10^6 SHEP-2 and SHEP-21N cells contained equivalent amounts of protein (data not shown). Protein synthesis rates were analyzed by ^{35}S-methionin

incorporation. No differences were observed between SHEP-2 and N-myc expressing SHEP-21N cells. Also manipulation of the N-myc expression in SHEP-21N in a time course experiment did not reveal any difference in protein synthesis rates (data not shown). This suggests that the protein synthesis rate in SHEP-21N is either limited by a factor not induced by N-myc, or that protein synthesis is already maximal in the SHEP neuroblastoma cell line and beyond a level that can be boosted by N-myc.

SAGE libraries of neuroblastomas with and without amplification of endogenous N-myc

The SHEP neuroblastoma cell line has no endogenous N-*myc* expression, therefore the N-*myc* transfected cells do not necessarily have a genetic background representative for N-*myc* amplified neuroblastomas. For example, 90% of the N-*myc* amplified neuroblastomas have deletions of the chromosomal region 1p35-36 (Caron *et al.*, 1993), while the SHEP-2 and SHEP-21N cells have two apparently intact p arms of chromosome 1 (data not shown). To address the question whether the here identified downstream pathway of N-myc is also *in vivo* activated, we generated SAGE libraries of two neuroblastomas. Neuroblastoma tumor N159 has N-*myc* amplification and expression and neuroblastoma N52 is an N-*myc* single copy tumor without N-*myc* expression (Fig. 5B, lanes 9 and 10). We sequenced 39.598 tags of the two libraries. The tag frequencies were normalized per 20,000 tags and compared. N-*myc* was represented by 16 tags in N159 and 0 tags in N52. There are 52 tags differentially expressed ($p<0.01$) in the libraries. These differences are probably only partly caused by N-myc, as the two tumors are likely to differ in more aspects. We analyzed which of the N-myc target genes identified in the SHEP cells did correlate with N-myc in the two tumors.

The 56 significantly ($p<0.01$) induced ribosomal protein genes detected in SHEP-21N produce a total of 988 tags in N52 and 1600 tags in N159 (per 20.000 tags). The N-*myc* amplified N159 tumor therefore has a 62% higher

ribosomal protein gene expression. There are 36 tags with an increase of at least 50% and 22 tags with an increase of at least 100% in N159 compared to N52 (Figure 2B). These increases are more moderate than in the SHEP-21N cells (compare Fig. 2A and 2B), but strongly suggest that N-myc induces ribosomal protein gene expression *in vivo*. Also other genes functioning in protein synthesis are upregulated in N159. Increased expression in N159 compared to N52 is seen for nucleophosmin (from 4 to 19.2 tags), nucleolin (3 to 9 tags), eukaryotic translation initiation factor 4A, isoform 1 (4 to 9 tags) and the translation elongation factors EEF1a1 (50 to 96 tags) and EEF1g (18.4 to 32.8 tags). There is almost no induction of the genes involved in glycolysis and oxidative phosphorylation. The expression levels of five representative genes were confirmed by hybridization of Northern blots with total RNA from N159 and N52 (Fig. 5B and data not shown). These results show that the expression levels of many of the N-myc target genes identified in the SHEP-21N cells are also *in vivo* correlated with N-*myc* amplification and overexpression. However, this does not hold for all genes, suggesting that other factors modulate the activity of N-myc target genes.

N-myc target gene expression analyzed in panels of neuroblastoma cell lines and tumors

To further analyze the induction of N-myc downstream genes in neuroblastoma, we examined their expression in a panel of neuroblastoma cell lines and tumors. Hybridization of a Northern blot of total RNA from 21 neuroblastoma cell lines showed a fair albeit imperfect correlation between expression of N-*myc*, nucleolin, nucleophosmin and the ribosomal protein PPARP0 (Figure 5A). Cell line SJNB12 has no N-*myc* expression, but a very high expression of the N-myc target genes. However, this cell line has c-*myc* amplification and over-expression (Figure 5A, lane 7 and Cheng *et al.*, 1995), suggesting that c-myc may induce the same target genes as N-myc (see below).

As cell lines are not fully representative for neuroblastoma tumors *in vivo*, we analyzed 16 fresh neuroblastomas including the aggressive stages 3 and 4 and the less aggressive stages 1, 2 and 4s. A Northern blot analysis showed a fair overall correlation between expression of N-*myc*, nucleolin and nucleophosmin (Figure 5B). There are some exceptions, but the overall results suggest that nucleolin and nucleophosmin are also *in vivo* targets of N-myc induction. Ribosomal protein S6 (RPS6) expression showed a less consistent relationship with N-*myc*, indicating that besides N-myc also other factors may modulate its expression.

Several N-myc target genes are induced or suppressed by c-*myc* as well N-*myc* belongs to the same family of proto-oncogenes as c-*myc*. Since both oncogenes induce similar phenotypic effects and share several target genes, we analyzed whether the N-myc downstream targets identified in this study are targets of c-*myc* as well. We therefore analyzed the melanoma cell line IGR39D and a c-*myc* transfected clone of this cell line (clone 3, Versteeg *et al.*, 1988). Northern blots with total RNA of these cell lines were hybridized with the 26 probes tested on the SHEP-2 and SHEP-21N cells. Nine of 23 N-myc induced targets appeared to be induced by c-*myc* as well (Figure 6). They are the ribosomal protein genes S12, S27, S19, S6 and nucleolin, nucleophosmin, ubiquitin, GAPDH and NDUFB4. Three of the N-myc-suppressed targets were tested and found to be suppressed by c-myc as well. They were Osteonectin (Table 1, No. 118/123/125), Plasminogen activator inhibitor type 1 (Table 1, No. 112) and connective tissue growth factor (Table 1, No 127). Therefore, c-myc and N-myc share about 46% of their target genes in the here tested cell systems. Interestingly, nucleophosmin, nucleolin and most ribosomal protein genes are among them.

We found induction of 86 transcripts contributing to ribosome biogenesis, mRNA translation, protein maturation and protein turnover, demonstrating

that enhancement of protein synthesis is a major function of N-myc. We found a striking 45% higher rRNA content in SHEP-21N than in SHEP-2. There was no overall increase in the rate of protein synthesis in SHEP-21N. One interpretation is that some rate limiting components of the protein synthesis machinery are not induced in SHEP-21N cells. The SAGE libraries of the N-*myc* single copy neuroblastoma N52 and the N-*myc* amplified tumor N159 showed that ribosomal protein genes, nucleolin and nucleophosmin and five translation initiation and elongation factors are over-expressed in the N-*myc* amplified neuroblastoma *in vivo*. The Northern blot analysis of 37 neuroblastomas and neuroblastoma cell lines further confirmed induction of these genes in N-myc amplified neuroblastoma. These results show that myc genes function as major regulators of protein synthesis. This is in line with the reduced rate of protein synthesis in fibroblasts with a homozygous inactivation of c-*myc* (Mateyak et al., 1997) and the increased protein synthesis in fibroblast after activation of c-myc (Scmidt, 1999).

Energy production, mitochondria and apoptosis

The two other comprehensive sets of N-myc downstream target genes are implicated in the glycolysis and the mitochondrial electron transfer and ATP synthesis pathway. The identification of the electron transfer and ATP synthesis pathway as a major target of N-myc induction bears on the relationship between the mitochondrial transmembrane potential and apoptosis. Mitochondria have two faces: they provide the energy for fast cycling cells and they can drive the cell into apoptosis. Similarly, the myc oncogenes can induce vigorous cell proliferation as well as massive apoptosis. N-myc expression renders SHEP-21N cells susceptible to apoptotic triggers (Fulda et al., 1999; Lutz et al, 1998). Many key events in apoptosis focus on mitochondrial membrane potential (reviewed in Green and Reed, 1998). Examples are cytochrome c release, hyperpolarization of the inner membrane, opening of the permeability transition pore and generation of reactive oxigen

species (ROS). During normal electron transport in the mitochondrial membrane, 1 to 5% of the electrons lose their way and generate ROS. Any interruption of the electron transfer pathway strongly increases ROS production, with a deleterious effect on the cell (Kroemer et al., 1997). Enhancement of the electron flow by N-myc would upon interruption of the electron transfer chain boost ROS production. In addition, the moderate up-regulation of VDAC (Figure 1) could stimulate cytochrome c release and apoptosis. Therefore, N-myc induction of the electron transfer genes logically provides the energy required for cell proliferation. Meanwhile, it could increase the deadly potential of the mitochondria and upon triggering tip the scale towards execution of apoptosis.

Interestingly, tags for oxidative phosphorylation pathways are not over-expressed in the N-*myc* amplified N159 tumor. This tumor might have been selected *in vivo* for additional defects, that interfere with part of the N-myc downstream pathway. While SHEP-21N cells expressing N-myc are susceptible to apoptotic triggers (Lutz et al., 1998), neuroblastoma cell lines with overexpression of endogenous N-*myc* are refractory to such triggers. This shows that these cell lines have defects in the pro-apoptotic arm of the N-myc downstream pathway. It will be interesting to analyze whether this relates to the lack of induction of mitochondrial protein genes.

N-*myc* and c-*myc* share target genes

To date, only two target genes of N-myc have been published, which are targets of c-myc as welL (Lutz et al., 1996; Eilers et al., 1991; Bello-Fernadez et al., 1993). Of the 23 upregulated targets of N-myc that we tested on Northern blots, 9 are induced by c-*myc* in transfected melanoma cells. Both down-regulated N-myc targets that we tested were also downregulated by c-myc. Since the N-myc induced downstream pathway genes form very concise functional groups of genes, we postulated that N-myc functions as a general stimulator of protein synthesis and energy production. Since c-myc has an

equally powerful growth-inducing and transforming effect as N-myc, it is difficult to envisage that c-myc would only induce a subset of the genes that are necessary to boost the protein and ATP synthesis machinery's. It appears more likely that N-myc and c-myc activate the same basic cellular functions. Indeed, c-myc is implicated in induction of protein synthesis in fibroblasts cell lines (see above). We observed that induction of genes by N-myc strongly depends on their basic expression levels (fig.2). It is therefore possible that high expression of potential target genes in the original melanoma cell line may have prevented their induction by c-myc.

The physiological role of myc genes has been enigmatic, as only very few target genes were identified thus far. Here we describe 351 transcript tags that identify 335 genes defined by their unigene number that are target of N-myc or potential target of N-myc, some of which are target of c-myc as well. These results show that myc genes function as major regulators of protein synthesis and cellular energy production. It is likely that this induction mediates the enhanced transition through the G1 phase of the cell cycle in normally proliferating cells and in cells that are induced to proliferate by physiological stimuli. The effect on protein synthesis confirms earlier postulations based on the identification of a limited set of target genes (Schmidt, 1999, Mateyak et al., 1997, Johnston et al., 1999). The stimulatory effect on genes in the electron transfer and ATP synthesis pathway is unexpected and fits well with the energy requirements for enhanced protein production and G1 transition and could relate to the well established apoptotic effect of *myc* genes.

List of tags and genes induced or suppressed by N-myc

Table 1 lists all tags that we found to be significantly ($p<0.01$) induced or suppressed by N-myc in the comparison of the SHEP-2 and SHEP-21N SAGE libraries. The comparison is base on 21,559 tags of SHEP-21N and 44,674 tags

of SHEP-2. The tag frequencies shown are normalized per 10.000 tags (column SHEP-2 and SHEP-21N). The column "ratio ON:OFF" shows the fold induction (positive values) or suppression (negative values) by N-myc. When a tag had a zero expression in one of the libraries, we assumed for ratio calculation that the tag was present one time in the entire library. The Unigene numbers of the National Center for Biotechnology Information (NCBI, Bethesda, USA) are given in the column "Unigene". The numbers are based on the NCBI Unigene database as by 29-3-2000. The next column shows the Unigene description. Furthermore, for each Unigene cluster, one or two Genbank accession codes are given. For some tags, we identified two possible corresponding genes. This is indicated by an asterix in the column next to the tag.

Table 2: this table lists tags that were identified to differ significantly ($p < 0.01$) between the SAGE libraries of SHEP-2 and SHEP-21N after extending the sequencing of library SHEP-21N from 21,559 tags to 34,426 tags. The table lists expression levels in both libraries expressed per 20,000 transcript tags (column 'SHEP-2' and 'SHEP-21N'), the unigene number as identified by the computer program described by Caron et al. (2001) and in some cases a Genbank accession number of a clone corresponding to the Unigene cluster.

Experimental Procedures

Cell lines

Neuroblastoma cell lines and culture conditions were as described before (Cheng *et al.*, 1995). The melanome cell lines IGR39D and clone 3 were described by Versteeg *et al.* (1988). The SHEP cell lines were maintained in RPMI 1640 medium supplemented with 10% fetal calf serum, 4 mM L-glutamine, 100U/ml penicillin and 100 µg/ml streptomycin (Lutz *et al.*, 1996). Tetracycline (Sigma) was used at a concentration of 10 ng/ml medium to inhibit N-*myc* expression.

Generation of SAGE libraries

SAGE was performed as described by Velculescu *et al.* (1995) with a few adaptations. Total RNA was extracted by guanidium thiocyanate (Chromczynski and Sacchi, 1987). Poly(A)$^+$ RNA was isolated using the MessageMaker kit (Gibco/BRL) according to the manufacturer's instructions. SAGE libraries were generated using minimally 4 μg poly(A)$^+$ RNA. The cDNA was synthesized according to the Superscript Choice System (Gibco/BRL), digested with *Nla*III and bound to streptavidine-coated magnetic beads (Dynal). Linkers containing recognition sites for *Bsm*FI were ligated to the cDNA. Linker tags including a cDNA tag were released by *Bsm*FI digestion, ligated to one another and amplified. The PCR products were heated for 5 min at 65^{0}C before preparative analysis on a polyacrylamide gel. After the ligation into concatameres a second heating step was included (15 min at 65^{0}C) and fragments between 800bp and 1500bp were purified and cloned in pZero-1 (Invitrogen). Colonies were screened with PCR using M13 forward and reverse primers. Inserts larger than 300bp were sequenced with a BigDye terminator kit and analyzed on a 377 ABI automated sequencer (Perkin Elmer).

Analysis SAGE database

The SAGE libraries were analyzed using the SAGE 300 program software package (Velsculescu *et al.*, 1997). P-values were calculated using Monte Carlo simulations.Transcripts were identified by comparison of the tags in the database with the "tag to gene map" (SAGEmap) from Cancer Genome Anatomy Project at the NCBI (http://www.ncbi.nlm.nih.gov/SAGE). This database links Unigene clusters to SAGE tags (Lal *et al.*, 1999). The gene assignments were subsequently checked by hand for sequencing errors causing incorrect tags and for erroneous gene assignments based on hybrid Unigene clusters. Other database analyses and generation of specific primers utilized the Wisconsin GCG package software.

Northern Blot analysis

Total RNA (20 μg per lane) was electrophoresed through a 0.8% agarose gel in the presence of 6.7 % formaldehyde and blotted on Hybond N membranes (Amersham) in 10 x SSC. Hybridization was carried out overnight in 0.5 M $NaHPO_4$, pH 7.0, 7 % SDS, 1 mM EDTA at 65°C. Filters were washed in 40 mM $NaHPO_4$, 1% SDS at 65°C. Probes were labelled by random priming of sequence-verified PCR products. A complete list of all the primers used in RT-PCR reactions is available on request.

Total Protein content

Exponentially growing cells were harvested and cell number was determined using a Coulter counter. Cells (1 x 10^6) were lysed in 20 mM Tris-HCl (pH 8.0), 137 mM NaCl, 10% glycerol, 1% NP40 and protease inhibitors (protease cocktail, Roche). Samples were assayed with the Bio-Rad Protein assay. Assays were performed at least in duplicate.

Western Blots

Cell lysates were separated on SDS-polyacrylamide gel and electroblotted onto Immobilon-P transfer membrane (Millipore). Blocking of the membrane and incubation with antibodies involved standard procedures. Proteins were visualized using the ECL detection system (Amersham). Anti-nucleophosmin monoclonal antibody was a gift of dr. P.K. Chan (Baylor College of Medicine). The antibody against nucleolin was a gift of Dr. P. Bouvet (CNRS, IPBS, Toulouse, France). Anti-N-myc was obtained from PharmIngen (Clone B8.4.B).

Total rRNA content

Total RNA of 1 x 10^6 exponentially growing cells was extracted by guanidium isothiocyanate (Chromczynski and Sacchi, 1987) and photospectrometrically quantified. Results of ten isolations of each of the cell lines SHEP-2 and

SHEP-21N were statistically analyzed with the Students T test for independent samples. Aliquots on a per cell basis were subjected to agarose gel electrophoresis and stained with ethidium bromide. The relative fluorescence of the rRNA bands was quantified using the Kodak Digital Science 1D Image Analysis Software package (EDAS 120).

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Legends to the figures:

Figure 1. Northern blot analysis of N-myc downstream target genes. Equal amounts of total RNA from exponentially growing SHEP-2 and SHEP-21N cells were loaded. Northern blots were hybridized with probes for the 23 indicated N-myc targets.

Figure 2. Level of induction of the 56 ribosomal protein genes identified as N-myc targets ($p<0.01$) in SHEP-21N cells. A. Fold induction by N-myc in SHEP-21N as a function of the basic expression levels in SHEP-2. X-coordinate: basic expression level in SHEP-2 normalized per 10.000 tags. Y-coordinate: fold induction in SHEP-21N. B. Increase of the same 56 ribosomal protein genes in N-*myc* amplified neuroblastoma N159 as a function of the basic expression level in N-*myc* single copy neuroblastoma N52. X coordinate: expression level in N52 normalized per 10.000 tags. Y coordinate: Fold increase in N159 relative to N52.

Figure 3. Time-course analysis of N-*myc* and downstream target gene induction in SHEP-21N cells. SHEP-21N cells were treated for 24 hours with 10ng/ml tetracycline, washed and grown for an additional 36 hours without tetracycline. Cells were harvested at 0 hr, 8hr and 24 hr of tetracyclin treatment. Subsequent samples were taken at 1 hr, 2 hr, 4 hr, 8 hr, 10 hr, 12 hr, 24 hr and 36 hr after removal of the antibiotic. A: Northern blot analysis of total RNA at indicated time points. B: Western blot analysis of N-myc protein at indicated time points. Ten mg of total protein samples of the time-course experiment were fractionated through a 10% SDS-PAGE gel, blotted on Immobilon membrane and probed with a monoclonal anti-N-myc antibody.

Figure 4. Nucleolin and Nucleophosmin protein expression and total RNA content of SHEP-2 and SHEP-21N. A: Western blot analysis of nucleolin, N-MYC and nucleophosmin protein expression. Total cell extracts (10 μg) were fractionated through an acrylamide gel, blotted and probed with polyclonal antibodies against nucleolin (upper panel) and monoclonal antibodies against N-myc (middle panel) and nucleophosmin (lower panel). Control cell lines IMR32 and SK-N-FI have high, respectively low expression of N-*myc*, nucleolin and nucleophosmin. B: Total RNA content of SHEP-2 and SHEP-

21N. RNA was isolated from ten samples of 10^6 cells of each cell line and photospectromerically analyzed. Error bars give the S.D.

Figure 5. Northern blot analysis of total RNA from neuroblastoma cell lines and tumors. Filters were hybridized with indicated probes. RNA quantification was checked by ethidium bromide staining, the 28S band is shown. A: panel of 21 neuroblastoma cell lines. B: Panel of 16 fresh tumors. Tumors in lanes 1-9 are N-myc amplified.

Figure 6. Northern blot analysis of induction of N-myc target genes in a c-myc-transfected melanoma cell line. Clone 3 is a c-myc transfected clone of the IGR39D melanoma cell line. Equal amounts of total RNA of IGR39D and clone 3 were loaded. Filters were hybridized with the indicated probes.

TABLE 1 MYCN regulated genes (grouped by functional category) 10-4-00

							RIBOSOMAL PROTEINS	
TC			47,7	0,000	0,0	10,7	6174	ribosomal protein S17	AA876041, AI564812,
CC			43,5	0,000	0,0	9,7	180920	ribosomal protein S9	AI064904, U14971
AG			39,3	0,000	0,4	17,2	82146	ribosomal protein S12	AA483128, AA524764,
CA			29,0	0,000	0,2	6,5	75456	ribosomal protein L18	L11566, AA513721,
C			26,9	0,000	0,2	6,0	178391	L44-like ribosomal protein (L44L), Homo sapiens Bruton's tyrosine kinase (BTK), alpha-D-gal	W05120, AA181201,
G			20,7	0,000	0,7	13,8	6102	ribosomal protein S20	AI741180, AA054902,
CA			16,6	0,000	0,4	7,4	165590	ribosomal protein S13	AI350382, AI290903,
GG			16,6	0,000	0,9	14,8	80617	ribosomal protein S16	AI282556, AA628076,
GG			12,4	0,006	0,2	2,8	3254	ribosomal protein L23-like	U28596, Z49254,
C			12,4	0,006	0,2	2,8	1948	ribosomal protein S21	AW195829
CA			12,4	0,000	0,7	8,3	179666	ribosomal protein L35a	AW328435, AW328458,
CA			11,4	0,000	0,4	5,1	74267	ribosomal protein L15	AW327407, AA669521,
CT			11,4	0,000	1,3	15,3	73742	ribosomal protein, large, P0	AA807794, AI174682,
TA			11,0	0,000	3,1	34,3	182426	ribosomal protein S2	X17206, AA704039,
G			9,9	0,000	2,2	22,3	178551	ribosomal protein L6	AW327732, AI200056,
GA			9,2	0,000	1,6	14,4	153177	ribosomal protein S28	AA229774, AA421061,
A			8,3	0,010	0,0	1,9	128400	ESTs, Highly similar to 60S RIBOSOMAL PROTEIN L39 [H.sapiens]	AA075869
GA			8,0	0,000	1,6	12,5	180450	ribosomal protein S24	AW410149, D51704,
G			7,7	0,000	2,2	17,2	75362	ribosomal protein S18	AA179543, AW408851,
AA			7,4	0,000	1,6	11,8	2017	ribosomal protein L38	AA669304, AA747113,
AA			7,1	0,000	1,6	11,1	91379	ribosomal protein L26	AA084377, AA111974,
CA			6,8	0,000	1,1	7,4	99914	ribosomal protein L22	AA420829, AA603500,
TG			6,0	0,000	1,8	10,7	157650	ribosomal protein L9	D14531, U09953,
GC			5,9	0,000	3,1	18,6	182825	ribosomal protein L35	AA457581, AA479946,
GA			5,9	0,000	2,7	15,8	119598	ribosomal protein L3	AA121930, AW022193,
TG			5,7	0,002	0,9	5,1	158675	ribosomal protein L14	AA857488, AA188409,
GT			5,7	0,000	8,3	46,8	183698	ribosomal protein L29	U10248, U49083,
GG			5,3	0,000	4,9	26,0	183593	ribosomal protein L18a	AI719134, AA047273,
GT			5,2	0,000	4,0	20,9	195453	ribosomal protein S27 (metallopanstimulin 1)	AA525023, AA555149,
AG			5,1	0,000	2,5	12,5	252259	ribosomal protein S3	AA706649, AI792490,
T			4,8	0,000	2,0	9,7	75536	ribosomal protein S7	M77233, AA513485,
GC			4,7	0,000	2,7	12,5	177415	ribosomal protein S30; Finkel-Biskis-Reilly murine sarcoma virus (FBR-MuSV) ubiquitously e	AA025283, AA055838,
CC			4,7	0,000	1,8	8,3	76879	ribosomal protein L19	AI219423, AA075893,
G			4,6	0,000	2,2	10,2	180946	ribosomal protein L5	W95799, AA491764,
C			4,3	0,000	21,5	91,4	151004	ribosomal protein S8	AI357743, AW051116,
CC			4,1	0,000	5,4	22,3	4737	ribosomal protein L28	AA225930, AA480489,
GG			4,0	0,000	3,1	12,5	184014	ribosomal protein L31	AA340384, AA024502,
GA			4,0	0,000	7,6	30,1	180842	ribosomal protein L13	AI503685, AI992115,

39	CTGTTGGTGA		3,9	0,000	4,0	15,8	3463	ribosomal protein S23	AW020385, AW022474,
40	AGGGCTTCCA		3,8	0,000	5,1	19,5	29797	ribosomal protein L10	AA244126, AA508385,
41	TAAGGAGCTG		3,8	0,000	3,8	14,4	77904	ribosomal protein S26	AW022428, X89654,
42	GTGAAGGCAG		3,7	0,000	3,4	12,5	77039	ribosomal protein S3A	AA070817, AA074191,
43	CGCCGGAACA		3,4	0,000	3,8	13,0	286	ribosomal protein L4	AL119264, AW021149,
44	AGGCTACGGA		3,4	0,000	8,7	29,7	119122	ribosomal protein L13a	AA190364, AW328422,
45	CCTTCGAGAT		3,3	0,000	4,0	13,5	76194	ribosomal protein S5	AI971757, AA057826,
46	AAGACAGTGG		3,3	0,000	9,2	30,1	184109	ribosomal protein L37a	AA353080, AW020311,
47	TTACCATATC		3,3	0,000	6,9	22,7	177461	ribosomal protein L39	AW023857, T40220,
48	TGTGCTAAAT		3,2	0,000	5,1	16,7	250695	ribosomal protein L34	AW020381, N95940,
49	GCCGTGTCCG		3,2	0,001	2,9	9,3	241507	ribosomal protein S6	AA148285, AA167421,
50	GGCAAGAAGA		3,0	0,000	3,8	11,6	111611	ribosomal protein L27	AA531226, AA885989,
51	ACATCATCGA		3,0	0,000	7,4	22,3	182979	ribosomal protein L12	L06505, AA464263,
52	GGATTTGGCC		2,9	0,000	16,8	48,2	251247	ribosomal protein, large P2	AA037465, AA064832,
53	CGCTGGTTCC		2,8	0,000	8,5	24,1	179943	ribosomal protein L11	AA226392, AA230322,
54	TACAAGAGGA		2,8	0,005	2,7	7,4	174131	ribosomal protein L6	AI042169, D17554
55	TTGGTCCTCT		2,7	0,000	18,4	50,1	108124	ribosomal protein L41	Z12962, AA046319,
56	CTCCTCACCT		2,6	0,001	4,9	13,0	119122	ribosomal protein L13a	AW242158, AW245840,
57	AATAGGTCCA		2,5	0,000	9,2	23,2	113029	ribosomal protein S25	AA228760, AA228897,
58	GAGGGAGTTT		2,5	0,000	18,6	46,8	76064	ribosomal protein L27a	AA228189, AA229849,
59	AAGAAGATAG		2,5	0,002	4,3	10,7	184776	ribosomal protein L23a	AA040728, AA088894,
60	ATTATTTTTC		2,5	0,006	3,6	8,8	153	ribosomal protein L7	AA136446, AW020191,
61	ACTCCAAAAA		2,2	0,000	10,3	22,7	133230	ribosomal protein S15	AA078683, AA151459,
	SECTION 2.							PROTEIN SYNTHESIS	
62	CTGGCGAGCG		16,6	0,000	0,2	4,2	174070	ubiquitin carrier protein	AA211097, AA283711,
63	GAGCGGGATG		16,6	0,001	0,2	3,7	77060	proteasome (prosome, macropain) subunit, beta type, 6	D29012, X61971,
64	GCATAGGCTG		12,4	0,000	0,4	5,6	12084	Tu translation elongation factor, mitochondrial	AL037768, L38995,
65	GGCTCCCACT		11,7	0,000	0,7	7,9	74335	heat shock 60kD protein 1, beta	AW023752, AA034511,
66	GCCCAGCTGG		11,4	0,000	0,4	5,1	223241	eukaryotic translation elongation factor 1 delta (guanine nucleotide exchange protein)	Z21507, AA489523,
67	TGAAATAAAC		10,4	0,004	0,0	2,3	173205	nucleophosmin (nucleolar phosphoprotein B23, numatrin)	AI184619
68	TACCAGTGTA		10,4	0,004	0,0	2,3	79037	heat shock 60kD protein 1 (chaperonin)	AW021205, AW103323,
69	TGTGTTGAGA		9,4	0,000	5,5	52,4	181165	eukaryotic translation elongation factor 1 alpha 1	AA630271, AA668532,
70	TGGGCAAAGC		9,0	0,000	2,0	18,1	2188	eukaryotic translation elongation factor 1 gamma	AA088923, AA190762,
71	ACATCCTCAC		8,3	0,010	0,0	1,9	18700	proteasome (prosome, macropain) 26S subunit, non-ATPase, 13	AA024939, AA149127,
72	TAAAATTTGT		8,3	0,010	0,0	1,9	93379	eukaryotic translation initiation factor 4B	AA054750, AA133563,
73	CAGTCTAAAA		8,3	0,003	0,4	3,7	76118	ubiquitin carboxyl-terminal esterase L1 (ubiquitin thiolesterase)	X04741, AA029783,
74	GAAGGCATCC		8,3	0,010	0,0	1,9	250758	proteasome (prosome, macropain) 26S subunit, ATPase, 3	F32284, AA130329,
75	TGGCTAGTGT		6,9	0,001	0,7	4,8	1°8965	proteasome (prosome, macropain) subunit, beta type, 7	AA804284, AA629875,
76	CAGATCTTTG		5,8	0,000	1,1	6,5	1.18502	ubiquitin A-52 residue ribosomal protein fusion product 1	AF075321, AI110823,

TABLE 1 MYCN regulated genes (grouped by functional category)

No	Tag Sequence	[illegible]	[illegible]	P value	SHEP-2	SHEP-21N	Unigene	Unigene description	Accession code
77	TCACAAGCAA		4,4	0,001	1,6	7,0	146763	nascent-polypeptide-associated complex alpha polypeptide	AF054187, X80909,
78	AACTCTTGAA		3,8	0,008	1,3	5,1	58189	eukaryotic translation initiation factor 3, subunit 3 (gamma, 40kD)	U54559, AA024720,
79	AGCACCTCCA		3,5	0,000	9,0	31,5	75309	eukaryotic translation elongation factor 2	AA229607, AA533837,
80	AGCCCTACAA	•	3,4	0,000	15,4	52,9	180532	heat shock 90kD protein 1, alpha	AW088888 , AW128905
81	CGCCGCGGTG		2,9	0,004	2,7	7,9	4835	eukaryotic translation initiation factor 3, subunit 8 (110kD)	AA573953, AA778285,
82	TTCATACACC	•	2,6	0,000	31,3	82,6	180532	heat shock 90kD protein 1, alpha	AW080884
83	TGAAATAAAA		2,5	0,000	10,1	25,5	173205	nucleophosmin (nucleolar phosphoprotein B23, numatrin)	AI928289, AW166850,
84	ATCAGTGGCT		2,5	0,009	3,1	7,9	89545	proteasome (prosome, macropain) subunit, beta type, 4	R25997, R68878,
85	CCCTGATTTT		-7,1	0,000	9,8	1,4	183684	eukaryotic translation initiation factor 4 gamma, 2	U73824, U76111,
86	AAGAGGTTTG		-9,2	0,001	4,3	0,0	74368	transmembrane protein (63kD), endoplasmic reticulum/Golgi intermediate compartment	X69910, AI131495,
87	GTTTTTGCTT		2,4	0,075	1,6	3,7	79110	Nucleolin	AA133588, AA135423, A
88	TACAAAACCA		2,1	0,923	0,9	1,9	79110	Nucleolin	AA098423, AA284953, A
	SECTION 3.							GENES INVOLVED IN METASTASIS	
89	AATAGAAATT		14,5	0,000	0,0	3,2	313	secreted phosphoprotein 1 (osteopontin, bone sialoprotein I, early T-lymphocyte activation 1)	AW021049, AA021512,
90	ATGCTCCCTG		14,5	0,000	0,0	3,2	79339	lectin, galactoside-binding, soluble, 3 binding protein (galectin 6 binding protein)	L13210, X79089
91	ACAGGGTGAC		14,5	0,030	0,0	3,2	174050	endothelial differentiation-related factor 1	AA975055, AA992919,
92	AACGCGGCCA		12,8	0,030	1,1	14,4	73798	macrophage migration inhibitory factor (glycosylation-inhibiting factor)	AA523321, AA927284
93	AAGAAAGGAG		11,4	0,000	0,9	10,2	202097	procollagen C-endopeptidase enhancer	L33799
94	GTAAGTCTCA		8,3	0,010	0,0	1,9	211584	neurofilament, light polypeptide (68kD)	AW022557, AA330627,
95	GCCGATCCTC		8,3	0,010	0,0	1,9	24930	tubulin-specific chaperone a	AF038952
96	GGCTCCTGGC		8,3	0,010	0,0	1,9	5215	integrin beta 4 binding protein	AF022229, AF047433,
97	GCCGGGTGGG		7,6	0,001	0,7	5,1	74631	basigin	D45131, L10240,
98	GGGGAAATCG		5,9	0,000	3,8	22,3	76293	thymosin, beta 10	AA147289, AA155816,
99	GCCCCCAATA		-1,7	0,007	20,1	11,8	227751	lectin, galactoside-binding, soluble, 1 (galectin 1)	J04456, X14829,
100	ACCAAAAACC		-1,8	0,003	21,3	11,8	172928	collagen, type I, alpha 1	AA454809, AA454820,
101	GTTGTGGTTA		-2,2	0,000	30,0	13,9	75415	beta-2-microglobulin	AB021288, AA897072,
102	AACTGCTTCA		-2,5	0,002	12,8	5,1	11538	actin related protein 2/3 complex, subunit 1B (41 kD)	AA031434, AA045773,
103	TCTCTGATGC		-2,7	0,001	12,5	4,6	6441	tissue inhibitor of metalloproteinase 2	AL110197, AL134962,
104	TGTACCTGTA		-2,8	0,000	14,1	5,1	169476	tubulin, alpha, ubiquitous	AA018607, AA018827,
105	GCTTTATTTG		-2,9	0,000	15,9	5,6	180952	actin, beta	AA419273, AA528145,
106	TCCTGTAAAG		-3,1	0,007	7,2	2,3	74034	caveolin 1, caveolae protein, 22kD	AA034380, AA044994,
107	TGCTAAAAAA		-4,7	0,000	17,6	3,7	146550	myosin, heavy polypeptide 9, non-muscle	AA041529, AA070528,
108	TGGAAATGAC		-4,8	0,000	42,8	8,8	172928	collagen, type I, alpha 1	AA853342, AA436411,
109	TGGAAATGCC		-5,0	0,001	6,8	1,4	172928	collagen, type I, alpha 1	AI190987, AI339782,
110	GTTTTTTTTA	•	-5,5	0,004	5,1	0,9	179573	collagen, type I, alpha 2	AI825593
111	ACAGGCTACG		-6,0	0,002	5,6	0,9	75777	transgelin	AI188763, AI540294,
112	TAAAAATGTT		-6,3	0,000	17,5	2,8	82085	plasminogen activator inhibitor, type I	M14083, AA040151,
113	GTTTCTAATA		-6,3	0,006	2,9	0,0	239298	microtubule-associated protein 4	AA043400, AA096288,
114	TTAAAGATTT		-7,1	0,000	13,2	1,9	77899	tropomyosin 1 (alpha)	AA026384, AA036782,

TABLE 1 MYCN regulated genes (grouped by functional category)

[illegible]	Tag sequence	[illegible]	[illegible]	P value	[illegible]	[illegible]	Unigene	Unigene description	Accession numbers
115	GACCGCAGGA		-7,2	0,003	3,4	0,0	119129	collagen, type IV, alpha 1	X03983, AW020005,
116	TAATCCTCAA		-9,2	0,000	7,6	0,9	78409	collagen, type XVIII, alpha 1	AA009987, AA099281,
117	TGTAGAAAAA		-9,2	0,004	4,3	0,6	119076	tubulin, beta polypeptide	AI308800, AI823884,
118	TTAGTGTCGT		-10,6	0,002	4,9	0,5	111779	secreted protein, acidic, cysteine-rich (osteonectin)	AW020685, AW022769,
119	GCCCCAATAA	*	-11,1	0,000	5,1	0,0	227751	lectin, galactoside-binding, soluble, 1 (galectin 1)	AA095630
120	AAAGTCATTG	*	-11,1	0,001	5,1	0,5	77899	tropomyosin 1 (alpha)	M19713
121	TGCAATATGC		-12,5	0,000	5,8	0,0	750	fibrillin 1 (Marfan syndrome)	L13923, X63556,
122	ATCTTGTTAC		-14,5	0,000	6,7	0,5	118162	fibronectin 1	AW020421, AW020447,
123	AAAAAGCTGC		-15,9	0,000	7,4	0,0	111779	secreted protein, acidic, cysteine-rich (osteonectin)	AA987875, AI755199,
124	AAAATATTTT		-18,8	0,000	8,7	0,0	119000	actinin, alpha 1	X55187, AA024695,
125	ATGTGAAGAG		-19,9	0,000	268,2	13,5	111779	secreted protein, acidic, cysteine-rich (osteonectin)	AA229382, AA852763,
126	TGGCCTAATA		-27,5	0,000	12,8	0,0	1501	syndecan 2 (heparan sulfate proteoglycan 1, cell surface-associated, fibroglycan)	AA873519, AI279414,
127	TTTGCACCTT		-37,6	0,000	17,5	0,5	75511	connective tissue growth factor	AW021964, U14750,
	SECTION 4.							GLYCOLYSIS ENZYMES	
128	TAGCTTCTTC		14,5	0,000	0,0	3,2	76392	aldehyde dehydrogenase 1, soluble	AA911016, AI150648,
129	ACCTTGTGCC		10,4	0,004	0,0	2,3	878	sorbitol dehydrogenase	AA570172, AA570189,
130	TCTGCTTGTC		10,4	0,004	0,0	2,3	76392	aldehyde dehydrogenase 1, soluble	H79748, H05271,
131	TGACTGAAGC		10,4	0,004	0,0	2,3	3343	3-phosphoglycerate dehydrogenase	AA742550, AA113268,
132	TGGCCCCACC		7,5	0,000	1,1	8,3	195281	pyruvate kinase, muscle	AA769601, AA769285,
133	GCGACCGTCA		7,3	0,000	0,9	6,5	183760	aldolase A, fructose-bisphosphate	AA169782, AA557193,
134	TACCATCAAT		4,7	0,000	5,8	27,4	195188	glyceraldehyde-3-phosphate dehydrogenase	AA226858, AA522734,
135	TGAGGGAATA		4,4	0,000	2,2	9,7	83848	triosephosphate isomerase 1	AA587096, AA587188,
	SECTION 5.							MITOCHONDRIAL FUNCTIONAL PROTEINS	
136	GAATCGGTTA		22,8	0,000	0,2	5,1	80595	NADH dehydrogenase (ubiquinone) Fe-S protein 5 (15kD) (NDUFB4)	AF047434, AA457800,
137	GGGGGTCACC		18,6	0,000	0,0	4,2	80986	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit c (subunit 9), isoform 1	AA659764, AA886065,
138	AAGGAGTTTG		14,5	0,000	0,0	3,2	661	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 7 (18kD, B18)	AA493442, AA832435,
139	AGGTCCTAGC		14,5	0,000	0,4	6,5	226795	glutathione S-transferase pi	F34719, U30897,
140	CTTAGAGCCC		12,4	0,006	0,2	2,8	211929	thioredoxin, mitochondrial	AI680239
141	TTCTGGCTGC		12,4	0,006	0,2	2,8	119251	ubiquinol-cytochrome c reductase core protein I	F28971, AI005342,
142	GTGGTACAGG		7,3	0,007	0,4	3,2	31731	ESTs, Highly similar to PUTATIVE PEROXISOMAL ANTIOXIDANT ENZYME [H.sapiens]	AA745952, AA829707,
143	GTGACAACAC		5,2	0,003	0,9	4,6	149155	voltage-dependent anion channel 1	AA907238, AI076271,
144	GCCTGCTGGG		4,8	0,000	2,0	9,7	3708	glutathione peroxidase 4 (phospholipid hydroperoxidase)	X71973, AA743017,
145	GGGGACTGAA		4,6	0,003	1,1	5,1	3709	low molecular mass ubiquinone-binding protein (9.5kD)	AA010379, AA025093,
146	CCCATCGTCC	*	4,3	0,000	21,5	91,4	mito	cytochrome c oxidase II (Welle et al., 1999), Tag matches mitochondrial sequences	AA069405, AA074137
147	TGATTTCACT	*	4,1	0,000	4,0	16,7	mito	cytochrome c oxidase III (Welle et al., 1999), Tag matches mitochondrial sequences	AA757623
148	TGAAGGAGCC		4,1	0,007	1,1	4,6	89399	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit c (subunit 9), isoform 2	F27013, D13119,

TABLE 1 MYCN regulated genes (grouped by functional category)

[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	Unigene	Unigene description	Accession codes
149	GTGACCTCCT		4,1	0,003	1,3	6,6	81097	cytochrome c oxidase subunit VIII	F28061, F28676,
150	AGCCCTACAA	*	3,4	0,000	15,4	52,9	mito	NADH dehydrogenase 3 (Welle et al., 1999), Tag matches mitochondrial sequences	AA577697 , AI022799
151	TTCATACACC	*	2,8	0,000	31,3	82,6	mito	NADH dehydrogenase 4/4L (Welle et al., 1999), Tag matches mitochondrial sequences	AA971178
152	CACCTAATTG	*	2,3	0,000	34,2	79,3	mito	ATPase 6/8 (Welle et al., 1999), Tag matches mitochondrial sequences	AA031407
153	GAGAGCTCCC		-2,8	0,008	7,8	2,8	169919	electron-transfer-flavoprotein, alpha polypeptide (glutaric aciduria II)	AI364921
154	GTAAGATTAG	*	-5,8	0,000	8,1	1,4	5417	oxygen regulated protein (150kD)	AA828743
155	GCCCCAATAA	*	-11,1	0,000	5,1	0,0	173554	ubiquinol-cytochrome c reductase core protein II	AA425586
	SECTION 6.							OTHER GENES	
156	CCCCCTGGAT		21,8	0,000	0,4	9,7	N/A	calcyclin	AI832624, AA081048,
157	GTGCGCTAGG		18,6	0,000	0,2	4,2	9408	ESTs, classified into serine/threonine kinase. Highly similar to The KIAA0151 gene product is	AA420781, AA482367,
158	GAGTGGGGGC		18,6	0,000	0,2	4,2	14089	ESTs, Weakly similar to LYSOSOMAL PRO-X CARBOXYPEPTIDASE PRECURSOR [H.s	AA382125, AA419395,
159	TTTCCTTCCT	*	16,6	0,001	0,2	3,7	104143	clathrin, light polypeptide (Lca)	AA574409, AA737504,
160	ATAAGATACA		16,6	0,000	0,0	3,7	199026	RAS p21 protein activator (GTPase activating protein) 3 (Ins(1,3,4,5)P4-binding protein)	X89399
161	AACGCTGCCT		14,5	0,000	0,0	3,2	28914	adenine phosphoribosyltransferase	AA444061, AA535523
162	ATTTGTCCCA		12,4	0,000	0,4	5,6	139800	high-mobility group (nonhistone chromosomal) protein isoforms I and Y	AA071304, AA074087,
163	TCAGACGCAG		12,4	0,006	0,2	2,8	182371	prothymosin, alpha (gene sequence 28)	AW128309
164	TCTCTTTTTC	*	12,4	0,006	0,2	2,8	119529	epididymal secretory protein (19.5kD)	AA025798, AA039545,
165	TTTCAGGGGA	*	12,4	0,006	0,2	2,8	2853	poly(rC)-binding protein 1	AI312897
166	CGGCCCAACG		12,4	0,001	0,0	2,8	20521	HMT1 (hnRNP methyltransferase, S. cerevisiae)-like 2	AA031375, AA167773,
167	TTCTCCCGCT		12,4	0,001	0,0	2,8	118126	protective protein for beta-galactosidase (galactosialidosis)	M22960, AI752880,
168	GGGGGACGGC		12,4	0,001	0,0	2,8	21348	ESTs, Weakly similar to F42C5.7 gene product [C.elegans]	AA015954, AA028165,
169	GGCCCTGAGC		11,4	0,000	0,4	5,1	71618	polymerase (RNA) II (DNA directed) polypeptide L (7.6kD), Human RNA polymerase II subu	AA570105, AA662657,
170	TATGTGATTT		10,4	0,000	0,4	4,8	5216	ESTs, Highly similar to HSPC028 [H.sapiens]	AA713577, AA748622,
171	GGCAGAGGAC		10,4	0,000	0,7	7,0	118638	non-metastatic cells 1, protein (NM23A) expressed in	X17620, AA046312,
172	GCCAAGATGC		10,4	0,004	0,0	2,3	83135	p53-responsive gene 6	AA038471, AA149817,
173	CCGACACTAC		10,4	0,004	0,0	2,3	242024	guanine nucleotide binding protein (G protein), beta polypeptide 2	M16538, AA548194,
174	CCGTCATCCT		10,4	0,004	0,0	2,3	153591	Not56 (D. melanogaster)-like protein	AI914552, AA412507,
175	TGAAATAAAC	*	10,4	0,004	0,0	2,3	155212	methylmalonyl Coenzyme A mutase	AI569612, AI628986,
176	TGAAATAAAC	*	10,4	0,004	0,0	2,3	238380	Human endogenous retroviral protease mRNA, complete cds	AA527289
177	GACCCTGCCC		10,4	0,004	0,0	2,3	173464	FK506-binding protein 8 (38kD)	L37033, AA587607,
178	CAGCAGAAGC		9,8	0,000	0,9	8,8	256313	pinin, desmosome associated protein	N76807
179	CTCATAGCAG	*	9,3	0,001	0,4	4,2	103838	chromosome 1 open reading frame 9	AI312752, AI312755
180	CTCATAGCAG	*	9,3	0,001	0,4	4,2	119252	tumor protein, translationally-controlled 1	N92214, AA045631,
181	CTGGGCCTGG		8,3	0,010	0,0	1,9	74573	similar to vaccinia virus HindIII K4L ORF	N99342, D45708,
182	GGGGTAAGAA		8,3	0,010	0,0	1,9	80423	prostatic binding protein	AA613924, AA825254,
183	GGGCTGGGCC		8,3	0,010	0,0	1,9	100071	6-phosphogluconolactonase	AI217069, AI279087,
184	TTTTTTGTAA		8,3	0,010	0,0	1,9	97658	SH3-domain binding protein 1	AA748769
185	GCAGTGGCCT		8,3	0,010	0,0	1,9	184276	solute carrier family 9 (sodium/hydrogen exchanger), isoform 3 regulatory factor 1	AA625289, AA593821,
186	CCCCCAATGC		8,3	0,010	0,0	1,9	115232	Spliceosome protein SAP-62	AI280058, AI348200,

TABLE 1 MYCN regulated genes (grouped by functional category)

187	GTGCTGTGAT	*	8,3	0,010	0,0	1,9	1053	small nuclear ribonucleoprotein polypeptide C	AA099406, AA099919,
188	CAGTTGGTTG		8,3	0,010	0,0	1,9	155216	E1B-55kDa-associated protein 5	AA130531, AA155809,
189	ATCCATAGTG		8,3	0,010	0,0	1,9	68772	TATA box binding protein (TBP)-associated factor, RNA polymerase II, N, 66kD (RNA-bindin	AA682359, AA857343,
190	CTGGATGCCG		8,3	0,010	0,0	1,9	108061	RD RNA-binding protein	AA569818, AA988602,
191	CTGACCCCCT		8,3	0,010	0,0	1,9	26492	beta-1,3-glucuronyltransferase 3 (glucuronosyltransferase I)	AB009598, AJ005965,
192	CCTGTACCCC	*	8,3	0,010	0,0	1,9	32317	Sox-like transcriptional factor	AA045957, AA196459,
193	TAAAATTTGT	*	8,3	0,010	0,0	1,9	32317	Sox-like transcriptional factor	AA919117
194	TGGCCTGCCC		8,3	0,010	0,0	1,9	181002	MLL septin-like fusion	AA761307, AA831791,
195	TGGCCTCCCC		8,3	0,010	0,0	1,9	159161	Rho GDP dissociation inhibitor (GDI) alpha	X59350
196	TGCAGCGCCT		8,3	0,010	0,0	1,9	77573	uridine phosphorylase	X90858, AI016589,
197	TGAGGGGTGA		8,3	0,010	0,0	1,9	252979	G protein pathway suppressor 1	AA521025, AA569807,
198	TGAGGCCAGG	*	8,3	0,010	0,0	1,9	79162	structure specific recognition protein 1	AW328280, M86737,
199	GAGAGAAGAG		8,3	0,010	0,0	1,9	13476	UDP-Gal:betaGlcNAc beta 1,4- galactosyltransferase, polypeptide 3	AA721091, AA743639,
200	TCTTCTCACA	*	8,3	0,010	0,0	1,9	656	cell division cycle 25C	AA206499, AA534482,
201	GCCGCTACTT		8,3	0,010	0,0	1,9	32889	calcitonin receptor-like receptor activity modifying protein 1	AI951585, AJ001014
202	CCCTCCTCCG		8,3	0,010	0,0	1,9	81131	guanidinoacetate N-methyltransferase	O59710, AI123221,
203	TATGACCACA	*	8,3	0,010	0,0	1,9	6650	vacuolar protein sorting 45B (yeast homolog)	AA765998, AA769317,
204	TACATTCACC		8,3	0,010	0,0	1,9	82043	D123 gene product	D14878, U27112,
205	AAGCGGGACC		8,3	0,010	0,0	1,9	153436	N-acetyltransferase, homolog of S. cerevisiae ARD1	X77588, AA158247,
206	GATCAATGGA	*	8,3	0,010	0,0	1,9	3030	EphB1	AA449788, AA640161,
207	GATCAATGGA	*	8,3	0,010	0,0	1,9	251788	glucosamine-6-phosphate deaminase	AA031910, AA151758,
208	GCCGCCATCT		8,3	0,010	0,0	1,9	88643	transketolase (Wernicke-Korsakoff syndrome)	U55017
209	CCCTGGGTTC		7,7	0,000	2,5	19,0	111334	ferritin, light polypeptide	M11147, M12938,
210	CAGCCTTGGA		7,3	0,007	0,4	3,2	85348	RNA binding motif protein 8	AA045586, AA188855,
211	ACCCTTCCCT	*	7,3	0,007	0,4	3,2	89526	ESTs, Weakly similar to VON EBNER'S GLAND PROTEIN PRECURSOR [H.sapiens]	AA936288, AA977608,
212	GAGGGGAAAC		7,3	0,007	0,4	3,2	81972	SHC (Src homology 2 domain-containing) transforming protein 1	AA767916, X68148,
213	ACCCTTCCCT	*	7,3	0,007	0,4	3,2	74564	signal sequence receptor, beta (translocon-associated protein beta)	AW024384, D37991,
214	ACTGGGTCTA		6,6	0,000	1,6	10,2	250871	non-metastatic cells 2, protein (NM23B) expressed in	AA514798, AA828464,
215	TGGAGTGGAG		6,8	0,000	1,1	6,5	3764	guanylate kinase 1	F25667, AA024959,
216	CCCCTCCCTC	*	6,5	0,008	0,7	3,7	79410	solute carrier family 4, anion exchanger, member 2 (erythrocyte membrane protein band 3-li	AA741103, AA767203,
217	CCCCTCCCTC	*	6,5	0,008	0,7	3,7	74564	signal sequence receptor, beta (translocon-associated protein beta)	AW083845
218	ACAGTGGGGA		4,6	0,003	1,1	5,1	76839	zinc finger protein 6 (CMPX1)	AA740739, AA100383,
219	TGATTTCACT	*	4,1	0,000	4,0	16,7	24322	ATPase, H+ transporting, lysosomal (vacuolar proton pump) 9kD	AI065143
220	TTATGGGATC		4,0	0,000	3,4	13,5	5882	guanine nucleotide binding protein (G protein), beta polypeptide 2-like 1	M24194, AA480431,
221	ATAGACATAA		2,8	0,007	2,5	7,0	7-1814	complement component 1, q subcomponent binding protein	AI916184, AA195312,
222	CACCTAATTG	*	2,3	0,000	34,2	79,3	181368	U5 snRNP-specific protein (220 kD), ortholog of S. cerevisiae Prp8p	AW129234, AW151854,
223	GAAATACAGT	*	-1,8	0,006	18,6	10,2	79572	cathepsin D (lysosomal aspartyl protease)	AA046688, AA053376,
224	GCCTTCCAAT		-2,0	0,004	15,9	7,8	76053	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 5 (RNA helicase, 68kD)	AA080988, AW019938,
225	GTGTGTTTGT		-2,0	0,006	15,0	7,4	118787	transforming growth factor, beta-induced, 68kD	M77349, AW021500,
226	AGCAGATCAG		-2,2	0,004	13,2	6,0	119301	S100 calcium-binding protein A10 (annexin II ligand, calpactin I, light polypeptide (p11))	AW022967, AA009605,
227	CTGCCAAGTT		-2,6	0,003	10,3	3,7	76873	zyxin	AA040172, AA054721,
228	TTCTGTGAAT	*	-3,7	0,002	8,5	2,3	182183	caldesmon 1	AA552208, AA552209,

TABLE 1 MYCN regulated genes (grouped by functional category)

No.	Tag Sequence	[illegible]	Ratio	P value	[illegible]	[illegible]	Unigene	Unigene Description	Accession
229	CTTAATCCTG		-4,1	0,000	15,2	3,7	234433	ESTs, Weakly similar to transporter protein [H.sapiens]	AA664787, AW021494,
230	TCTCAATTCT	•	-4,5	0,003	6,3	1,4	173497	Sec23 (S. cerevisiae) homolog B	AI581164, AW135798,
231	GCCCTTTCTC		-4,5	0,000	12,6	2,8	7835	endocytic receptor (macrophage mannose receptor family)	AA126747, AA405572,
232	TCTCAATTCT	•	-4,5	0,003	6,3	1,4	N/A	BB1	AA410935, AA022590,
233	TTCTTGTTTT		-5,3	0,006	4,8	0,9	74621	prion protein (p27-30) (Creutzfeld-Jakob disease, Gerstmann-Strausler-Scheinker syndrome	D00015, M13667,
234	TATGACTTAA	•	-5,3	0,006	4,9	0,9	89230	potassium intermediate/small conductance calcium-activated channel, subfamily N, member	AA265078, AA491238,
235	GTTTTTTTTA	•	-5,5	0,004	5,1	0,9	10114	ESTs, Weakly similar to protein B [H.sapiens]	AA284721, AA496717,
236	GTCACAGTCC		-5,8	0,009	2,7	0,0	155321	serum response factor (c-fos serum response element-binding transcription factor)	AA024483, AA041538,
237	TAAGAAAATG		-6,3	0,008	2,9	0,0	75929	cadherin 11 (OB-cadherin, osteoblast)	AA258422, AA461076,
238	TTTTTTAAAA		-6,3	0,006	2,9	0,0	227400	mitogen-activated protein kinase kinase kinase kinase 3	AA043537
239	TTACTTATAC	•	-6,6	0,004	3,1	0,0	159	tumor necrosis factor receptor superfamily, member 1A	AW138089
240	TTACTTATAC	•	-6,6	0,004	3,1	0,0	28935	wingless-type MMTV integration site family, member 2B	AW129657
241	GCTGTTTTGT		-6,6	0,004	3,1	0,0	92186	KIAA0989 protein	AB023206, AA405541,
242	CTTTCTTTGA		-8,2	0,001	3,8	0,0	4909	regulated in glioma	AF052161, AA209468,
243	AAAAGATACT		-8,2	0,009	3,8	0,5	82071	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2	AA115949, AA146987,
244	TCCGTGGTTG		-8,2	0,001	3,8	0,0	79516	brain acid-soluble protein 1	AF039656, AA602987,
245	CATTATAACT		-8,2	0,001	3,8	0,0	84359	hypothetical protein	AA806434, AA832337,
246	TGTCATCACA		-9,2	0,004	4,3	0,5	83354	lysyl oxidase-like 2	AA126279, AA149435,
247	TTTTGTTTTG	•	-9,7	0,003	4,5	0,5	95583	transmembrane 4 superfamily member (tetraspan NET-7)	AA037844, AA040421,
248	TACAGAGGGA		-10,1	0,002	4,7	0,5	3776	zinc finger protein 216	AA730188, AA814563,
249	AAGTGAAACA		-11,1	0,001	5,1	0,5	93659	protein disulfide isomerase related protein (calcium-binding protein, intestinal-related)	AW411440
250	AGTTTCCCAA		-13,0	0,000	6,0	0,5	76854	SULT1C sulfotransferase	AF055584, AA113827,
251	TACAATAAAC		-14,0	0,000	6,5	0,0	9071	progesterone membrane binding protein	AA836144, AJ002030,
252	CATATCATTA		-15,9	0,000	7,4	0,0	119206	insulin-like growth factor binding protein 7	AL038223, AA745836,
	SECTION 7.							EST clones of unknown function	
253	GCACCTCAGC		14,5	0,000	0,0	3,2	13702	ESTs	AA027098, AA035781,
254	GAGAGAAAAT		14,5	0,000	0,0	3,2	181444	ESTs, Weakly similar to R12C12.6 [C.elegans]	AA476914, AA630706,
255	ACTTTTTAAA		14,1	0,000	1,1	15,8	157300	EST	AI365306
256	TTTCAGGGGA	•	12,4	0,005	0,2	2,8	3804	DKFZP564C1940 protein	AA831451, AA070917,
257	TGATGGGCAT		12,4	0,005	0,2	2,8	74284	ESTs, Moderately similar to Similar to S.cerevisiae hypothetical protein L3111 [H.sapiens]	AI888503, AI745361,
258	CTGGGCGTGT	•	12,4	0,001	0,0	2,8	109949	ESTs, Weakly similar to laminin beta-2 chain precursor [H.sapiens]	AA046403, AA759123,
259	ACGGTGATGT		12,4	0,001	0,0	2,8	10453	ESTs	AA005401, AA045808,
260	CTGGGCGTGT	•	12,4	0,001	0,0	2,8	15246	EST	T80794
261	TGAGGCCAGG	•	8,3	0,010	0,0	1,9	110128	ESTs	AA584384
262	GGTTTGTGTG		8,3	0,010	0,0	1,9	83954	Homo sapiens unknown mRNA	AF061739, AA732389,
263	TTGTGGGATC		8,3	0,010	0,0	1,9	167795	ESTs	AA018907, AA126950,
264	CCTGTACCCC	•	8,3	0,010	0,0	1,9	12342	Homo sapiens clone 24538 mRNA sequence	AI924428
265	TATGACCACA	•	8,3	0,010	0,0	1,9	176577	ESTs	AI651376
266	ACTACCTTCA		8,3	0,010	0,0	1,9	9601	ESTs, Highly similar to CGI-106 protein [H.sapiens]	AA744772, AA805690,

TABLE 1 MYCN regulated genes (grouped by functional category)

No.	[illegible]		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
287	CTGCTGTGAT	*	6,3	0,010	0,0	1,9	193909	ESTs, Weakly similar to !!!! ALU SUBFAMILY SC WARNING ENTRY !!!! [H.sapiens]	AA528209, AI690704,
288	GAGTGAGTGA	*	7,3	0,007	0,4	3,2	52186	ESTs, Weakly similar to !!!! ALU SUBFAMILY J WARNING ENTRY !!!! [H.sapiens]	H82203
289	GAGTGAGTGA	*	7,3	0,007	0,4	3,2	10463	ESTs, Weakly similar to C44C1.2 gene product [C.elegans]	AA019696, AA088449,
270	TTTGTTAAAA	*	4,1	0,007	1,1	4,6	111244	ESTs	AA748351, AA769191,
271	AAGATAATGC	*	4,1	0,007	1,1	4,6	102698	ESTs, Weakly similar to C11D2.4 [C.elegans]	AA808449, AA115697,
272	AAGATAATGC	*	4,1	0,007	1,1	4,6	251978	EST	C14037
273	TTTGTTAAAA	*	4,1	0,007	1,1	4,6	207118	EST	AI808514
274	AGGAAAGCTG		4,0	0,000	6,3	25,0	76437	DKFZP566B023 protein	AA523344, AA551988,
275	CAAGCATCCC		3,5	0,000	5,8	20,4	153423	ESTs	AW275649, AW276934,
276	GAAATACAGT	*	-1,8	0,006	18,6	10,2	87201	ESTs	AA122047, AA404659,
277	TTCTGTGAAT	*	-3,7	0,002	8,5	2,3	77870	ESTs	AA022926, AA121431,
278	GAAATAATGG	*	-4,0	0,001	9,2	2,3	178053	ESTs	AA412270, AI208372,
279	GAAATAATGG	*	-4,0	0,001	9,2	2,3	209037	ESTs	AI143898, AI808260
280	TATGACTTAA	*	-5,3	0,006	4,9	0,9	203352	ESTs	W52933
281	GTAAGATTAG	*	-5,6	0,000	8,1	1,4	250705	ESTs	AA953513, AI129290,
282	GCCATATTAT		-5,8	0,008	2,7	0,0	19260	KIAA0544 protein	R70600, AI081100,
283	AATTTTCATT	*	-6,3	0,006	2,9	0,0	77895	KIAA0008 gene product	AI889277
284	AATTTTCATT	*	-6,3	0,006	2,9	0,0	35092	ESTs	AA343561, AA449815,
285	TTCCTCCTTT	*	-7,7	0,002	3,6	0,0	226144	ESTs	AI215617, AI932565,
286	GCTTGTCTTT		-7,7	0,002	3,6	0,0	224520	ESTs	AI580377, AI953171
287	ACAGTGTTAA		-7,7	0,002	3,6	0,0	186342	ESTs	AA167571, AA653213,
288	TTCCTCCTTT	*	-7,7	0,002	3,6	0,0	115581	EST, Highly similar to KIAA0825 protein [H.sapiens]	AA463908
289	TTTTGTTTTG	*	-9,7	0,003	4,5	0,5	74667	ESTs	AA120954, AA489661,
290	ACAGATTTGA		-10,1	0,000	4,7	0,0	41271	ESTs	AA253217, AA599448,
291	TTCCCCCTTC		-10,6	0,000	4,9	0,0	163928	ESTs	AA577100, AI819034
292	AAAGTCATTG	*	-11,1	0,001	5,1	0,5	21145	Human BAC clone RG093M05 from 7q21-7q22	AI056386, AI161119
	SECTION 8.							UNIDENTIFIED TRANSCRIPTS	
293	CCGCCGAAGT		24,8	0,000	0,0	5,6			
294	ACCTTTTCAA		16,6	0,000	0,0	3,7			
295	GCCCCTCCGG		16,6	0,001	0,2	3,7			
296	GCTTTCTCAC		10,4	0,000	0,9	8,3			
297	CGCCGCGGCT		10,4	0,004	0,0	2,3			
298	GTGACCACGG		9,3	0,001	0,4	4,2			
299	GTTAACAGTC		6,3	0,010	0,0	1,9			
300	GCCGTTCTTA		6,3	0,010	0,0	1,9			
301	AGGCTACCGG		7,3	0,007	0,4	3,2			
302	ACTTTTTCAC		5,4	0,000	1,8	9,7			
303	TCCCCGTCAT		-4,5	0,003	6,3	1,4			
304	AGGAATGTTA		-5,1	0,008	4,7	0,8			

TABLE 1 MYCN regulated genes (grouped by functional category)

305	TCCCTTATTA		-5,3	0,005	4,9	0,9			
306	TCTTGATATT		-5,8	0,005	2,7	0,0			
307	AAGGCAATTT		-6,8	0,001	6,3	0,9			
308	TTCGGTTGGT		-7,2	0,000	10,1	1,4			
309	TCCCCGGTAC		-7,6	0,000	13,9	1,8			
310	GCTGACGTCA		-8,2	0,001	3,8	0,0			
311	TCCCCCGTAC		-8,7	0,001	4,0	0,0			
312	ACGTTCTCTT		-9,2	0,001	4,3	0,0			
313	TAACTTTTGG		-9,2	0,001	4,3	0,0			
314	AAATGCTTGG		-9,7	0,003	4,5	0,8			
315	CTAAAAACCT		-10,1	0,000	4,7	0,0			
316	GTGAGAGTTT		-12,1	0,000	5,6	0,8			
317	GGTGGACACG		-13,0	0,000	6,0	0,0			
318	TCCCCTATTA		-15,4	0,000	7,2	0,0			
319	CTTTATTCCA		-16,3	0,000	17,0	0,9			

No	Tag Sequence	SHEP 2	SHEP 2 IN	P value	Unigene cluster	Unigene description	Accession code
1	CGACGAGGAG	1.8	7.0	0.0087	9999	epithelial membrane protein 3	
2	TTTAAAAAAA*	21.7	7.0	0.0003	97437	centriole associated protein	
3	GAATAAATGT	7.2	1.2	0.0053	8762	FK506-binding protein 9 (63 kD)	AI088246
4	TAAAATAAAA*	5.0	0.0	0.0027	87100	ESTs	
5	TCTTCTGCCA	9.5	1.7	0.0021	86392	ESTs	
6	TGCTTTGGGA	1.4	5.8	0.0100	84344	ESTs, Highly similar to CGI-135 protein [H.sapiens]	AA788733
7	GGGCGCTGTG	0.9	6.4	0.0020	8372	ubiquinol-cytochrome c reductase (6.4kD) subunit	
8	TTAGATAAGC*	4.5	12.2	0.0036	82916	chaperonin containing TCP1, subunit 6A (zeta 1)	
9	AGCTCTCCCT	29.3	53.4	0.0000	82202	ribosomal protein L17	AA961432
10	CCCTGCCTTG	0.0	3.5	0.0052	82045	midkine (neurite growth-promoting factor 2)	
11	GCACAAGAAG	9.5	19.2	0.0037	81634	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit b, isoform 1	
12	TTAAAGGCCG	0.5	5.2	0.0025	79086	ribosomal protein, mitochondrial, L3	
13	AGAAAGATGT	9.5	20.3	0.0016	78225	annexin A1	AA846272
14	TTTAAAAAAA*	21.7	7.0	0.0003	77501	sarcoglycan, beta (43kD dystrophin-associated glycoprotein)	
15	GTAAAAAAAA*	44.7	20.9	0.0002	77495	UBX Domain-containing 1	AI492365
16	CTTGATTCCC	0.9	6.4	0.0020	77266	quiescin Q6	
17	TGTGCTCGGG	2.3	7.6	0.0091	76847	KIAA0088 protein	
18	CAGTCTCTCA	0.9	7.0	0.0010	76230	ribosomal protein S10	
19	TACGTACTGC	0.0	3.5	0.0052	76086	ESTs, Highly similar to small zinc finger-like protein [H.sapiens]	
20	GGCAAGCCCC	9.9	40.1	0.0000	76067	heat shock 27kD protein 1	AI540836
21	CGGCTGAATT	0.9	5.8	0.0043	75888	phosphogluconate dehydrogenase	
22	GGCTGGGGGC	19.4	38.3	0.0001	75721	profilin 1	AA903097
23	TGCTGGGTGG*	0.5	4.6	0.0056	754	folylpolyglutamate synthase	
24	TCAGATCTTT	39.7	70.9	0.0000	75344	ribosomal protein S4, X-linked	AA954645
25	AAGAGTTTTG	0.5	5.8	0.0010	75313	aldo-keto reductase family 1, member B1 (aldose reductase)	
26	ACAAATCCTT	1.8	7.0	0.0087	752	FK506-binding protein 1A (12kD)	
27	AATATGTGGG	5.4	12.8	0.0060	74649	cytochrome c oxidase subunit VIc	AI025170
28	GGGTTTTTAT	5.0	11.6	0.0100	74497	nuclease sensitive element binding protein 1	
29	TCTGCTTACA	2.7	8.1	0.0093	74267	ribosomal protein L15	
30	TTTAAAAAAA*	21.7	7.0	0.0003	74088	early growth response 3	
31	CAAACCATCC*	1.4	5.8	0.0100	65114	keratin 18	
32	CAGACTTTTG*	5.9	12.8	0.0100	63348	DKFZP586M121 protein	
33	TTGGGGTTTC	40.6	74.9	0.0000	62954	ferritin, heavy polypeptide 1	AA866040
34	AAGCCTTGCT	5.4	0.6	0.0098	6289	growth factor receptor-bound protein 2 [No, now described as small stress protein-like protein HSP22	AI039721
35	AAGAAACCTT	1.4	5.8	0.0100	5836	ESTs, Highly similar to CGI-138 protein [H.sapiens]	
36	AAGGAAATGA	4.5	0.0	0.0043	57929	slit (Drosophila) homolog 3	AI741785

37	AACTAAAAAA*	68.6	91.8	0.0010	55921	glutamyl-prolyl-tRNA synthetase	
38	ATAATTCTTT	73.1	102.8	0.0001	539	ribosomal protein S29	
39	GTAAAAAAAA*	44.7	20.9	0.0002	460	Activating Transcription Factor 3	AA903193
40	TTTTAAAAAT*	7.7	1.7	0.0100	45033	lacrimal proline rich protein	
41	CTCGAATAAA	4.5	0.0	0.0043	34871	KIAA0569 gene product	AI338485
42	AACTAAAAAA*	68.6	91.8	0.0010	3297	ribosomal protein S27a	
43	GAGGCGATCA	0.0	3.5	0.0052	30763	ESTs, Weakly similar to eyelid [D.melanogaster]	
44	GTGGCTGAAA	5.0	0.0	0.0027	29797	ribosomal protein L10	AI582446
45	GTTTCCCCAA*			0.0067	281434		
46	GGTGAAGACA	8.6	1.2	0.0015	26951	Human mRNA for KIAA0375 gene, complete cds	AI080611
47	GCATTTAAAT*	16.7	27.3	0.0065	250876	eukaryotic translation elongation factor 1 beta 2	
48	TTTTGTATTT	5.9	0.6	0.0055	250705	ESTs	
49	CAGACTTTTG*	5.9	12.8	0.0100	250501	TNF? elastin microfibril interface located protein	
50	GATCCCAACA	0.5	4.6	0.0056	25	ATP synthase, H+ transporting, mitochondrial F1 complex, beta polypeptide	
51	CTGTTGATTG	16.3	26.7	0.0070	249495	heterogeneous nuclear ribonucleoprotein A1	
52	CACGCAATGC*	0.5	5.8	0.0010	244	amino-terminal enhancer of split	AI015996
53	TGGTACACGT	0.9	5.8	0.0043	242463	keratin 8	
54	TTTTAAAAAT*	7.7	1.7	0.0100	240013	catechol-O-methyltransferase	
55	ATTCTCCAGT	48.8	72.6	0.0002	234518	ribosomal protein L23	
56	CCCTTAGCTT	13.5	3.5	0.0011	233936	myosin, light polypeptide, regulatory, non-sarcomeric (20kD)	AI033904
57	GTGGTGGGCG*	0.9	7.0	0.0010	233694	ESTs, Weakly similar to ZK1058.5 [C.elegans]	
58	GCGGGGTACC	1.4	5.8	0.0100	227823	pM5 protein	
59	GGAGAGTACA	0.0	3.5	0.0052	225939	sialyltransferase 9 (CMP-NeuAc:lactosylceramide alpha-2,3-sialyltransferase; GM3 synthase)	
60	TAAAATAAAA*	5.0	0.0	0.0027	21254	TRAF interacting protein	
61	GTGGTGGGCG*	0.9	7.0	0.0010	209741	EST	
62	GTGGTGGGCG*	0.9	7.0	0.0010	208985	ESTs	
63	TCCAAATCGA	6.8	1.2	0.0080	2064	vimentin	
64	TAAAATAAAA*	5.0	0.0	0.0027	204144	ESTs, Moderately similar to tumor necrosis factor-alpha-induced protein B12 [H.sapiens]	
65	TAAAATAAAA*	5.0	0.0	0.0027	202218	EST	
66	TTCAATAAAA*	58.7	93.0	0.0000	2012	transcobalamin I (vitamin B12 binding protein, R binder family)	AI738761
67	TGCTGGGTGG*	0.5	4.6	0.0056	198273	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 8 (19kD, ASHI)	
68	TAAAATAAAA*	5.0	0.0	0.0027	190401	ESTs, Weakly similar to predicted using Genefinder [C.elegans]	
69	AAGGTCGAGC	1.8	7.0	0.0087	184582	ribosomal protein L24	
70	GCATAATAGG	31.6	54.0	0.0001	184108	ribosomal protein L21	AI224420
71	GTAAAAAAAA*	44.7	20.9	0.0002	183842	Ubiquitin B	AA988706
72	TAATATTTTT	11.7	2.9	0.0020	182485	actinin, alpha 4	
73	GAAAAATGGT	48.3	87.7	0.0000	181357	laminin receptor 1 (67kD, ribosomal protein SA)	AI025931

74	AGAACCTTAA	5.0	0.0	0.0027	181244	major histocompatibility complex, class I, A	AI023950
75	CTCATAAGGA	49.2	70.9	0.0006	181165	eukaryotic translation elongation factor 1 alpha 1	
76	TAATTTTGGA	9.0	17.4	0.0078	180152	ESTs	
77	CAATAAATGT	37.0	57.5	0.0004	179779	ribosomal protein L37	
78	TTCAATAAAA*	58.7	93.0	0.0000	177592	ribosomal protein, large, P1	AA961386
79	TTAAATAGCA	6.8	0.6	0.0021	172928	collagen, type I, alpha 1	AA992596
80	GGAGGAGAGC	5.0	0.0	0.0027	172928	collagen, type I, alpha 1	
81	TGAAATTGTC	4.5	0.0	0.0043	172928	collagen, type I, alpha 1	
82	AATGCAGGCA	1.4	6.4	0.0067	172673	S-adenosylhomocysteine hydrolase	AI051370
83	TTCCGGTTCC	9.0	1.7	0.0030	172609	nucleobindin 1	AI025019
84	CACGCAATGC*	0.5	5.8	0.0010	170883	EST	AI015996
85	TAGACTTATT	0.5	4.6	0.0056	170197	glutamic-oxaloacetic transaminase 2, mitochondrial (aspartate aminotransferase 2)	
86	TTCACAGTGG	0.5	5.2	0.0025	169992	protein phosphatase 3 (formerly 2B), regulatory subunit B (19kD), alpha isoform (calcineurin B, type I)	
87	TGCACGTTTT	37.5	61.0	0.0001	169793	ribosomal protein L32	AA922605
88	GCAGCTCAGG	2.3	7.6	0.0091	167137	ESTs, Moderately similar to CATHEPSIN D PRECURSOR [H.sapiens]	
89	GGTGAGACAC	2.7	8.7	0.0071	164280	solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 6	
90	TTTAAAAAAA*	21.7	7.0	0.0003	155545	37 kDa leucine-rich repeat (LRR) protein	
91	ATGTCATCAA	1.4	7.0	0.0030	152936	adaptor-related protein complex 2, mu 1 subunit	
92	TAATAAAGGT	45.1	62.2	0.0029	151604	ribosomal protein S8	AI312878
93	TTAAAACAAA	4.1	0.0	0.0070	150508	ESTs	AI280093
94	TGGCCTAAAA	4.5	0.0	0.0043	1501	syndecan 2 (heparan sulfate proteoglycan 1, cell surface-associated, fibroglycan)	AI005403
95	GGTTAATTGA	4.1	0.0	0.0070	14376		AI590977
96	TTCAATAAAA*	58.7	93.0	0.0000	141269	ESTs	AA961386
97	ATGTGAAGAA	6.8	1.2	0.0080	13662	Homo sapiens clone 25036 mRNA sequence	AI279223
98	TTTAAAAAAA*	21.7	7.0	0.0003	134533	ESTs	
99	TTAGATAAGC*	4.5	12.2	0.0036	134350	ESTs	
100	GTGACAGAAG	5.4	13.4	0.0034	129673	eukaryotic translation initiation factor 4A, isoform 1	
101	CTGGGTTAAT	42.4	77.8	0.0000	126701	ribosomal protein S19	AA865047
102	CAAACCATCC*	1.4	5.8	0.0100	125170	ESTs	
103	GGGACTGGGC*	0.5	5.2	0.0025	122256	ESTs	
104	GCATTTAAAT*	16.7	27.3	0.0065	120979	ESTs	
105	TAGGTTGTCT	55.1	71.5	0.0057	119252	tumor protein, translationally-controlled 1	AA808956
106	GACGTGTGGG	0.0	4.6	0.0009	119192	H2A histone family, member Z	
107	GGCTTTACCC	9.0	20.9	0.0006	119140	eukaryotic translation initiation factor 5A	
108	AACTAATACT	4.5	12.8	0.0022	118724	DR1-associated protein 1 (negative cofactor 2 alpha)	AI028098
109	TAAAATAAAA*	5.0	0.0	0.0027	118246	ESTs	
110	GGGACTGGGC*	0.5	5.2	0.0025	117848	hemoglobin, epsilon 1	

111	TAAAAACAAA	5.4	0.6	0.0098	114599		AW029321, W1923
112	GTTTCCCCAA*	1.4	6.4	0.0067	112423	Homo sapiens mRNA; cDNA DKFZp586I1420 (from clone DKFZp586I1420)	
113	GCAAAAAAAA	67.3	31.4	0.0000	11221	ESTs, Weakly similar to fos39554_1 [H.sapiens]	AI057027
114	CCAGAACAGA	37.5	55.8	0.0011	111222	ribosomal protein L30	
115	GTACGGAGAT	0.5	4.6	0.0056	109225	vascular cell adhesion molecule 1	
116	GTTTCCCCAA*	1.4	6.4	0.0067	107573	sialyltransferase	
117	GGCCCCTCAC	0.5	4.6	0.0056	106283	insulin-like growth factor binding protein 6	
118	TTTAAAAAAA*	21.7	7.0	0.0003	106204	ESTs, Moderately similar to antigen containing epitope to monoclonal antibody MMS-85/12 [M.musculus]	
119	CCTCCCCCGT	0.9	5.2	0.0092	10488	Breakpoint cluster region protein, uterine leiomyoma, 1; barrier to autointegration factor	
120	AGCAGGGCTC	0.0	5.2	0.0004	100623	phospholipase C, beta 3, neighbor pseudogene	
121	AATTGCAAGC	5.0	0.0	0.0027	-		U50523
122	TTTAACGGCC	43.8	12.8	0.0000	-		AA196553
123	CATTGCCTTC	5.9	0.6	0.0055	-		
124	GCTTGCTGCC	4.5	0.0	0.0043	-		
125	AAAACATTCT	67.7	32.5	0.0000	-		AI538076
126	GCAGACATTG	0.9	5.8	0.0043	-		
127	ACCCTTGGCC	12.2	26.7	0.0003	-		
128	AGTAGGTGGC	8.1	1.7	0.0068	-	U5 snRNP-specific protein (220 kD), ortholog of S. cerevisiae Prp8p	
129	GCAAGCCAAC	16.7	33.1	0.0002	-	actinin, alpha 1	AI669642
130	TTAGCTTGTT	4.1	0.0	0.0070	-		
131	GTAATAACTT	4.1	0.0	0.0070	-		
132	CGCCGTCGGC	0.0	3.5	0.0052	-		
133	ACTCTTTCAA	0.5	4.6	0.0056	-		
134	TGCTACGAAA	5.9	0.6	0.0055	-		
135	CCCCGGTACA	7.2	1.2	0.0053	-		
136	TTATAAAAGA	10.8	3.5	0.0098	-		

Claims

1. A nucleic acid library comprising *myc*-dependent downstream genes or functional fragments thereof said genes essentially capable of supporting a neoplastic character of cancer such as growth, invasion or spread.

2. A library according to claim 1 wherein said *myc*-dependent downstream genes each comprises a nucleic acid essentially equivalent to a Tag sequence as shown in Table 1 or Table 2.

3. A library according to claim 1 or 2 wherein said myc-dependent downstream genes encode a ribosomal protein.

4. A library according to claim 1 or 2 wherein said myc-dependent downstream genes encode a protein related to protein synthesis.

5. A library according to claim 1 or 2 wherein said myc-dependent downstream genes encode a protein related to metastasis.

6. A library according to claim 1 or 2 wherein said myc-dependent downstream genes encode a glycolysis enzyme.

7. A library according to claim 1 or 2 wherein said myc-dependent downstream genes encode a mitochondrial functional protein.

8. A method for the treatment of cancer comprising modulating a *myc*-dependent downstream gene capable of supporting an essentially neoplastic character of said cancer.

9. A method according to claim 8 wherein said *myc*-dependent downstream gene comprises a nucleic acid functionally equivalent to a Tag sequence as shown in Table 1 or Table 2.

10. A method according to claim 8 or 9 wherein said cancer comprises *myc*-expressing tumour cells.

11. A method according to anyone of claims 8 to 10 wherein *myc* is N-*myc*.

12. A method according to claim 11 wherein said cancer comprises neuroblastoma.

13. A method for the diagnosis of cancer comprising detecting the relative presence or absence of a *myc*-dependent downstream gene capable of

supporting an essentially neoplastic character of said cancer or gene product derived thereof.

14. A method to enhance production of recombinant proteins comprising a protein production system with high expression of endogenous or transfected myc genes.

15. A method to identify a substance capable of interfering with n-myc or n-myc induced modulation of transcripts and/or proteins, comprising providing a cell with n-myc activity or a nucleic acid encoding n-myc activity and determining the modulation of said transcripts and/or proteins in the presence of said substances.

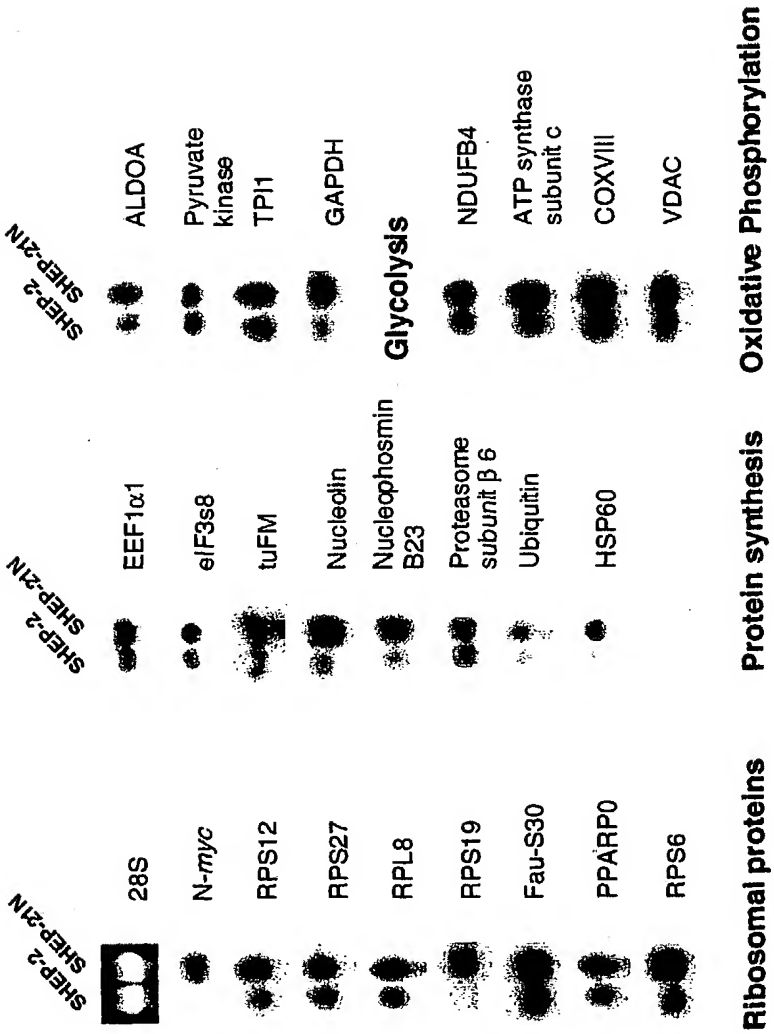
SHEP-2
SHEP-2tN
28S
N-myc
RPS12
RPS27
RPL8
RPS19
Fau-S30
PPARP0
RPS6
Ribosomal proteins
EEF1α1
eIF3s8
tuFM
Nucledin
Nucleophosmin B23
Proteasome subunit β 6
Ubiquitin
HSP60
Protein synthesis
ALDOA
Pyruvate kinase
TPI1
GAPDH
Glycolysis
NDUFB4
ATP synthase subunit c
COXVIII
VDAC
Oxidative Phosphorylation

Fig. 1

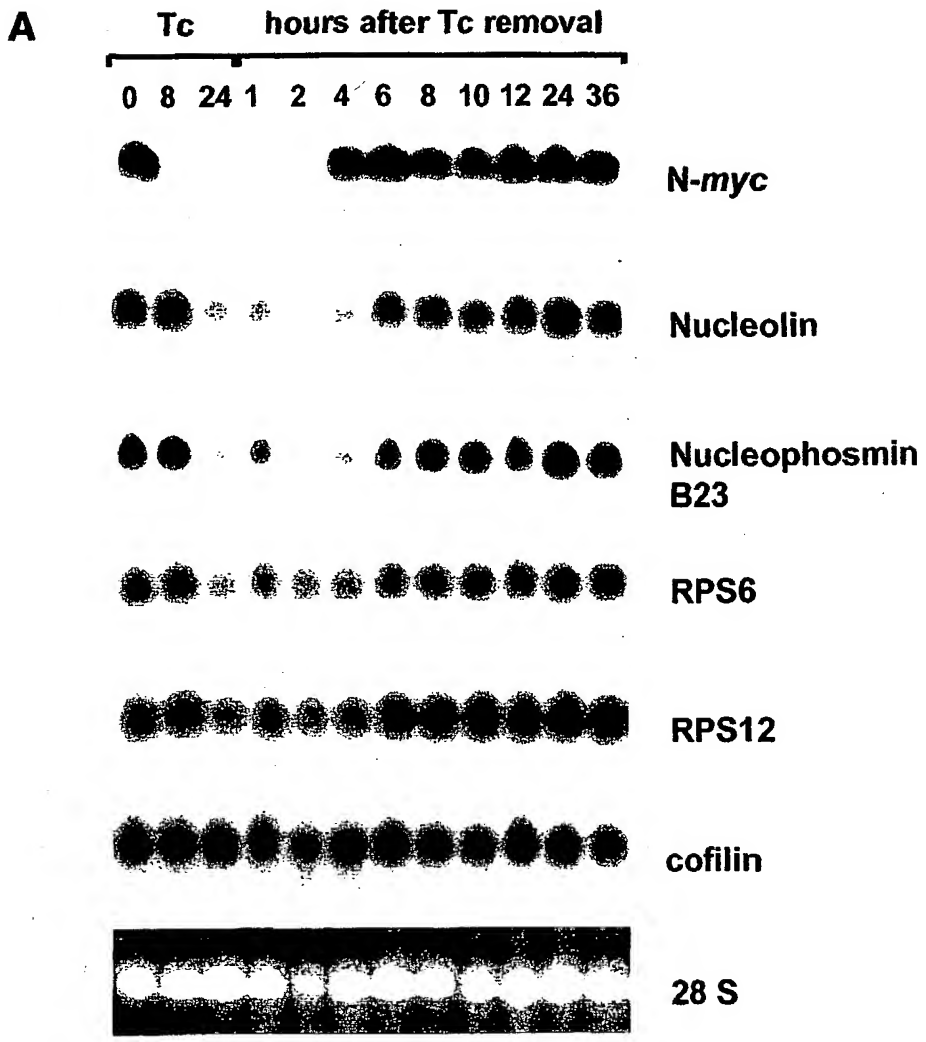
A
Tc
hours after Tc removal
0 8 24 1 2 4 6 8 10 12 24 36
N-myc
Nucleolin
Nucleophosmin B23
RPS6
RPS12
cofilin
28 S

Fig. 2A

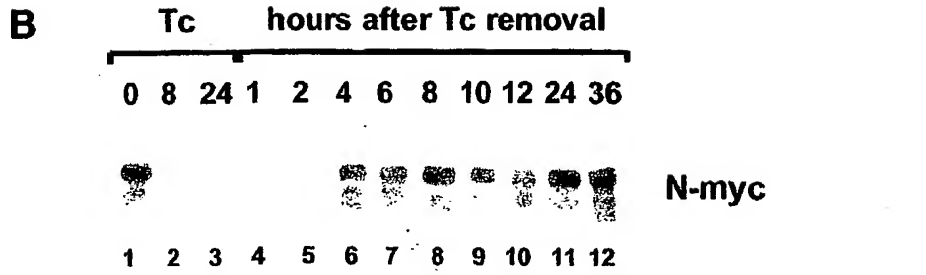
B
Tc
hours after Tc removal
0 8 24 1 2 4 6 8 10 12 24 36
N-myc
1 2 3 4 5 6 7 8 9 10 11 12

Fig. 2B

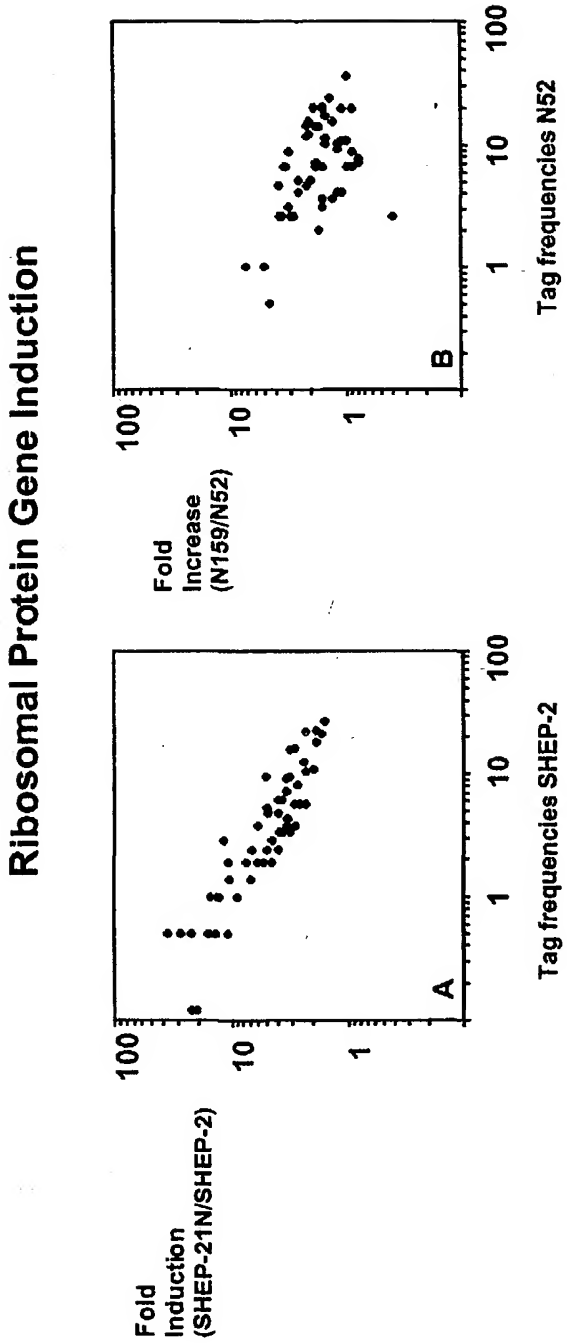
Ribosomal Protein Gene Induction
Fold Induction (SHEP-21N/SHEP-2)
Tag frequencies SHEP-2
Fold Increase (N159/N52)
Tag frequencies N52
A
B
100
10
1
1
10
100

Fig. 3

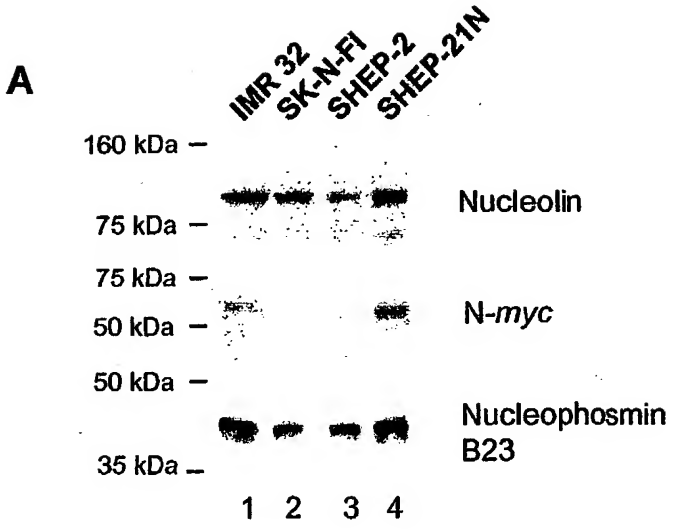
A
IMR 32
SK-N-FI
SHEP-2
SHEP-21N
160 kDa
75 kDa
75 kDa
50 kDa
50 kDa
35 kDa
Nucleolin
N-myc
Nucleophosmin
B23
1 2 3 4

Fig. 4A

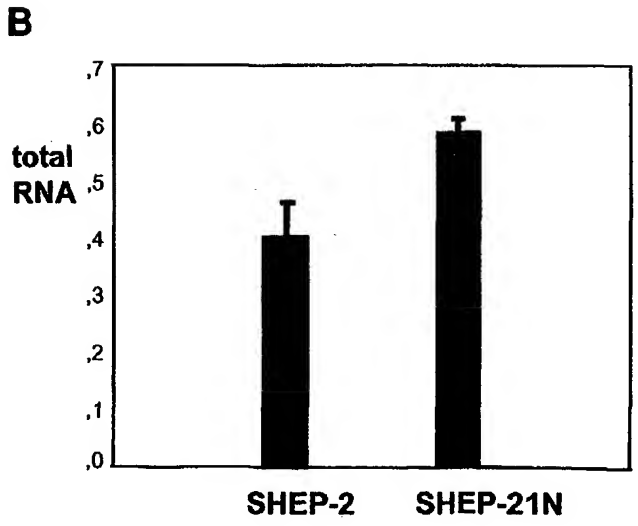
B
total
RNA
,7
,6
,5
,4
,3
,2
,1
,0
SHEP-2
SHEP-21N

Fig. 4B

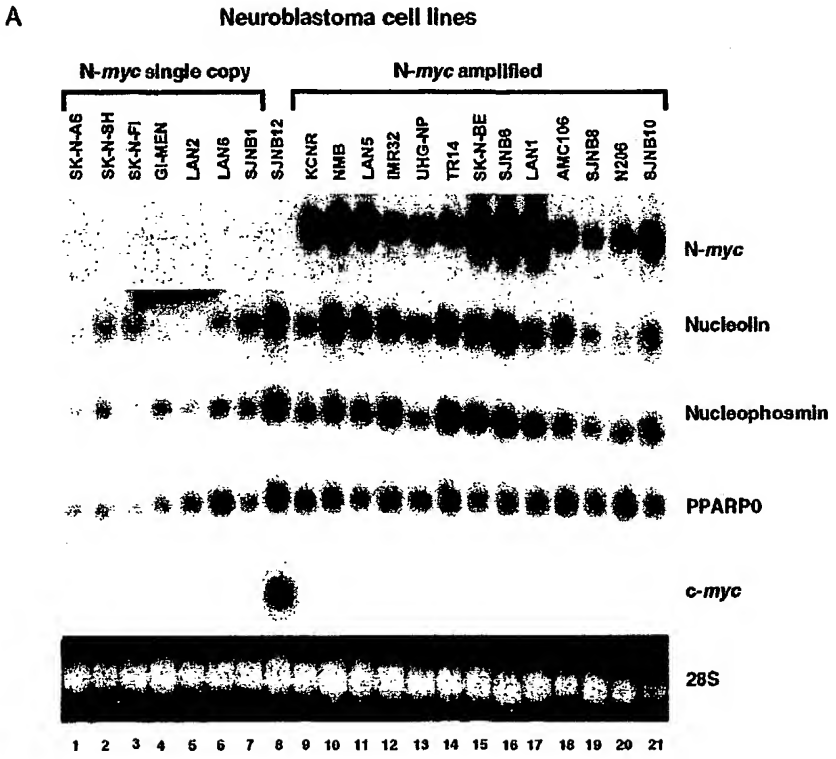
A
Neuroblastoma cell lines
N-myc single copy
N-myc amplified
SK-N-AS
SK-N-SH
SK-N-FI
GI-MEN
LAN2
LAN6
SJNB1
SJNB12
KCNR
NMB
LAN5
IMR32
UHG-NP
TR14
SK-N-BE
SJNB6
LAN1
AMC106
SJNB8
N206
SJNB10
N-myc
Nucleolin
Nucleophosmin
PPARP0
c-myc
28S
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21

Fig. 5A

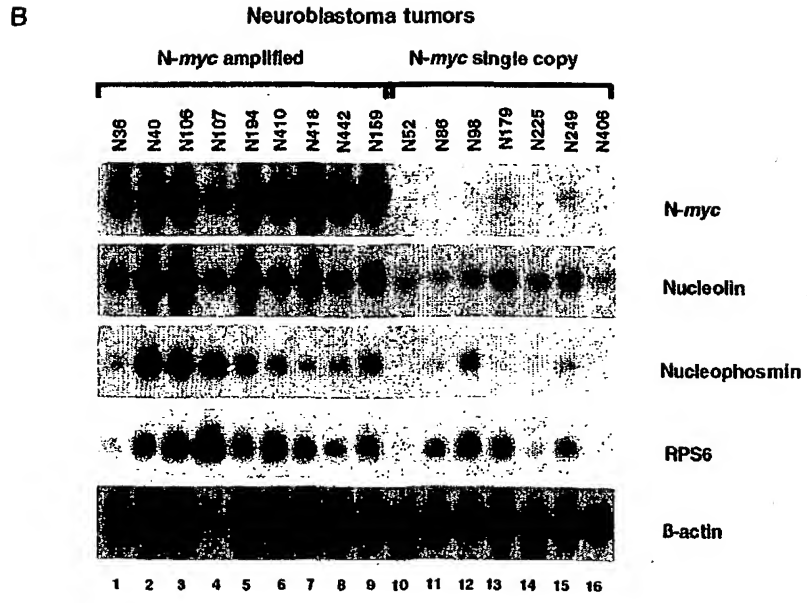
B
Neuroblastoma tumors
N-myc amplified
N-myc single copy
N38
N40
N106
N107
N194
N410
N418
N442
N159
N52
N88
N98
N179
N225
N249
N406
N-myc
Nucleolin
Nucleophosmin
RPS6
B-actin
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16

Fig. 5B

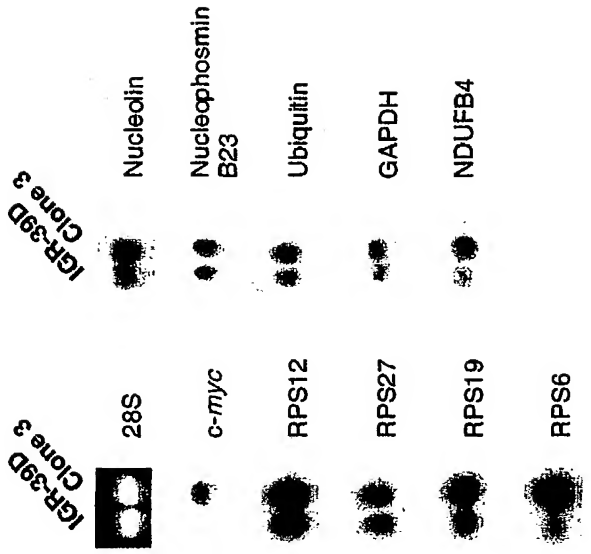
IGR-39D Clone 3
Nucleolin
Nucleophosmin B23
Ubiquitin
GAPDH
NDUFB4
IGR-39D Clone 3
28S
c-myc
RPS12
RPS27
RPS19
RPS6

Fig. 6